The Pathophysiology and Intensive Care of Acute Brain Injury: Analysis of Systemic and Intracranial Inflammatory Processes and the Evaluation of a Novel Therapy

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I hereby declare that this thesis has been composed by myself. All the work described herein was carried out by myself, in the Intensive Care Unit, Western General Hospital, Edinburgh between 1995 and 1997. Some of the work was carried out in collaboration with colleagues as detailed below.

Chapter 2 - Dr L Mascia (assistance with patient recruitment and blood sampling)
Mrs S Walker (assistance with RIA analysis, (ELISA analysis performed by author))

Chapter 3 - Miss J Monjardino (assistance with volunteer recruitment and data collection)

Chapter 4 - Dr L Mascia (assistance with cerebral blood flow measurement and data collection)

Mr D Signorini gave statistical advice.

Dr P J D Andrews oversaw the projects as my supervisor.

E G McKeating May 1997

ABSTRACT

Introduction - Outcome after acute injury to the brain depends on the survival of neurons. This requires an adequate supply of oxygen and metabolites to a cellular environment which supports cellular metabolic processes. The intracranial and systemic biochemical changes which occur after injury as a result of activation of inflammatory cascades may result in both reduced oxygen delivery and altered cellular metabolism. Therapeutic measures used in the intensive care unit to improve outcome after brain injury currently include monitoring of cerebral oxygenation and maintenance of cerebral perfusion by the use of pressor agents. Future management may include the use of antagonist drugs to biochemical inflammatory mediators, such as the cytokines or leucocyte adhesion molecules.

Methods - 32 patients admitted to the intensive care unit with either traumatic brain injury or spontaneous subarachnoid haemorrhage were studied. Paired arterial and jugular venous sera were taken at time points from admission until 4 days after injury and concentrations of cytokines, adhesion molecules, and the proteins neuron-specific enolase (NSE) and S-100 measured. Systemic concentrations and jugular venous-arterial gradients were studied in relation to each other, neurological outcome at 6 months, and other measures of injury type and severity. A comparison of two monitors (Critikon 2020 and Invos 3100) used to measure cerebral oxygenation by means of near infra-red spectroscopy in the intensive care unit was carried out using volunteer subjects. Assessment of the ability of each monitor to provide stable and consistent readings in subjects at rest was performed. The effect of controlled arterial hypertension on cerebral blood flow and metabolism was studied in 32 patients with acute brain injury in the intensive care unit.

Results - We found significant intracranial production of interleukin-6 after injury, and raised systemic concentrations of intercellular adhesion molecule-1 (ICAM-1), NSE and S-100. These systemic changes were all significantly related to outcome. Concentrations of ICAM-1 were negatively correlated with Glasgow Coma Score, but were not influenced by the presence of extracranial injuries. Systemic concentrations of L-selectin were significantly reduced after injury. The Critikon monitor failed to provide any reading in 44% of subjects, while the Invos failed in 20%. Differences between oxygen saturation readings from each monitor were related to the average saturation ($p<0.001$). Cerebral blood flow was adequate in all patients in the induced hypertension study. 58% of subjects were treated with a vasopressor to keep cerebral perfusion pressure above 70 mmHg. 33% of subjects developed cerebral hyperaemia, which was unrelated to vasopressor use.

Discussion - The highly significant relationship between systemic ICAM-1 and outcome suggests that antagonism of its effects may form part of future drug therapies for brain injury. The development of new monitoring technologies must result in monitors which function reliably in the intensive care unit, or their use will not be considered worthwhile. Induced hypertension is a treatment method which may reduce the likelihood of cerebral ischemia without resulting in inappropriate cerebral hyperaemia. Therapeutic measures which monitor and assist in cerebral oxygen delivery, and reduce inflammatory damage may improve outcome after brain injury.

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INTRODUCTION

Despite recent advances in the management of patients with acute injury to the brain, particularly in intensive care, those who have sustained a moderate to severe injury often have a dismally poor prognosis. Emphasis has previously been placed on the avoidance of systemic hypotension and hypoxia, but even in intensive care units where staff adhere rigorously to this approach, outcome appears to have improved little. A different therapeutic approach may therefore be required.

In the following thesis I shall review and discuss the significance of the inflammatory process in brain injury, in particular the parts played by the cytokines and leucocyte adhesion molecules, and the possibility that antagonism of their effects may form the basis of future drug therapy. This will be followed by a description of a study which examines the relationships between serial measurements of the cytokines and adhesion molecules in the serum of patients with brain injury, and relates these to injury severity and neurological outcome.

CHAPTER 1

A REVIEW OF THE CYTOKINES AND ADHESION MOLECULES IN ACUTE BRAIN INJURY

INTRODUCTION

The brain, once thought to be relatively shielded from the immunological and inflammatory processes which occur in the tissues of other body systems after acute injury, is in fact an active participant in these processes. Injury to the brain, as a result of trauma, haemorrhage or ischaemia, causes the release of mediators such as the cytokines, which activate inflammation and cause further secondary brain injury. Intensive care physicians can do little to alleviate the gravity of the primary injury, but by understanding the mechanisms responsible for secondary injury, and how these mechanisms may, in the future, be altered by drug therapy, they may be able to improve patient management and outcome.

A primary brain injury stimulates the cells of the central nervous system (CNS) to produce a variety of mediators. Laboratory and human studies have shown that there are at least three important cytokines which are released both by microglia and astrocytes after injury: interleukin-1β (IL-1β), tumour necrosis factor α (TNFα) and interleukin-6 (IL-6)[1-3].

These proteins, which function as intercellular communication molecules, seem to stimulate the reparative process which is termed gliosis. Gliosis, however, results in further production and release of cytokines by hypertrophied astrocytes and microglial cells, in addition to mediators released by cells of the peripheral immune system, such as polymorphonuclear cells, which migrate across a "leaky" blood-brain barrier. The net result may therefore be further damage to brain tissue. The first part of this review will focus on the parts played by these cytokines.

Leucocyte adhesion molecules, which are expressed on the surface of leucocytes and endothelial cells, control the migration of leucocytes into tissue. The expression of these molecules after brain injury is closely linked to cytokine production. They are thought to mediate toxicity in two ways: by causing leucocyte plugging of micro-vessels, and by facilitating the release of toxic superoxides by polymorphonuclear cells which migrate into brain tissue as a result of adhesion molecule activity. The second part of this review will focus on the parts played by the adhesion molecules intercellular adhesion molecule (ICAM)-1 , E-selectin, L-selectin and the integrins in brain injury, and on the evidence linking the cytokines to adhesion molecule upregulation. Finally I shall discuss whether anti-cytokine or anti-adhesion molecule therapy may improve outcome after acute brain injury.

THE CYTOKINES

The cytokines are low molecular weight polypeptides which are synthesized and released by multiple cells types throughout the body. Their actions are numerous but they act primarily as mediators of the inflammatory process (either pro- or anti-inflammatory) and as growth factors. Alternative names for the cytokines and adhesion molecules discussed in this review are listed in Table 1.1. Many of the clinical signs which are seen after an acute brain injury (eg pyrexia, neutrophilia and cerebral oedema, secondary to disruption of the blood-brain barrier) are believed to be caused by cytokine activity[4]. Cytokines also stimulate the release of many types of secondary mediators such as the superoxides, neuropeptides and arachidonic acid

Table 1.1 Alternative names for the cytokines and leucocyte adhesion molecules discussed in this review

Interleukin 1β	endogenous pyrogen
	leucocyte endogenous mediator
	lymphocyte activating factor
	mononuclear cell factor
	catabolin
Interleukin 6	hepatocyte-stimulating factor
	interferon β_2
	26 kD protein
	hybridoma growth factor
	B-cell stimulating factor
Tumour necrosis factor α	cachectin
L-selectin	endothelial leucocyte adhesion molecule-1 (ELAM-1)

derivatives, and upregulate the activity of the adhesion molecules. We shall consider the three cytokines IL-1β, TNFα and IL-6 separately in the following sections, but there is a great deal of overlap in the functions of these molecules - in particular between IL-1β and TNFα. Table 1.2 and Figure 1.1 summarise the systemic and CNS actions of these cytokines.

Interleukin 1β

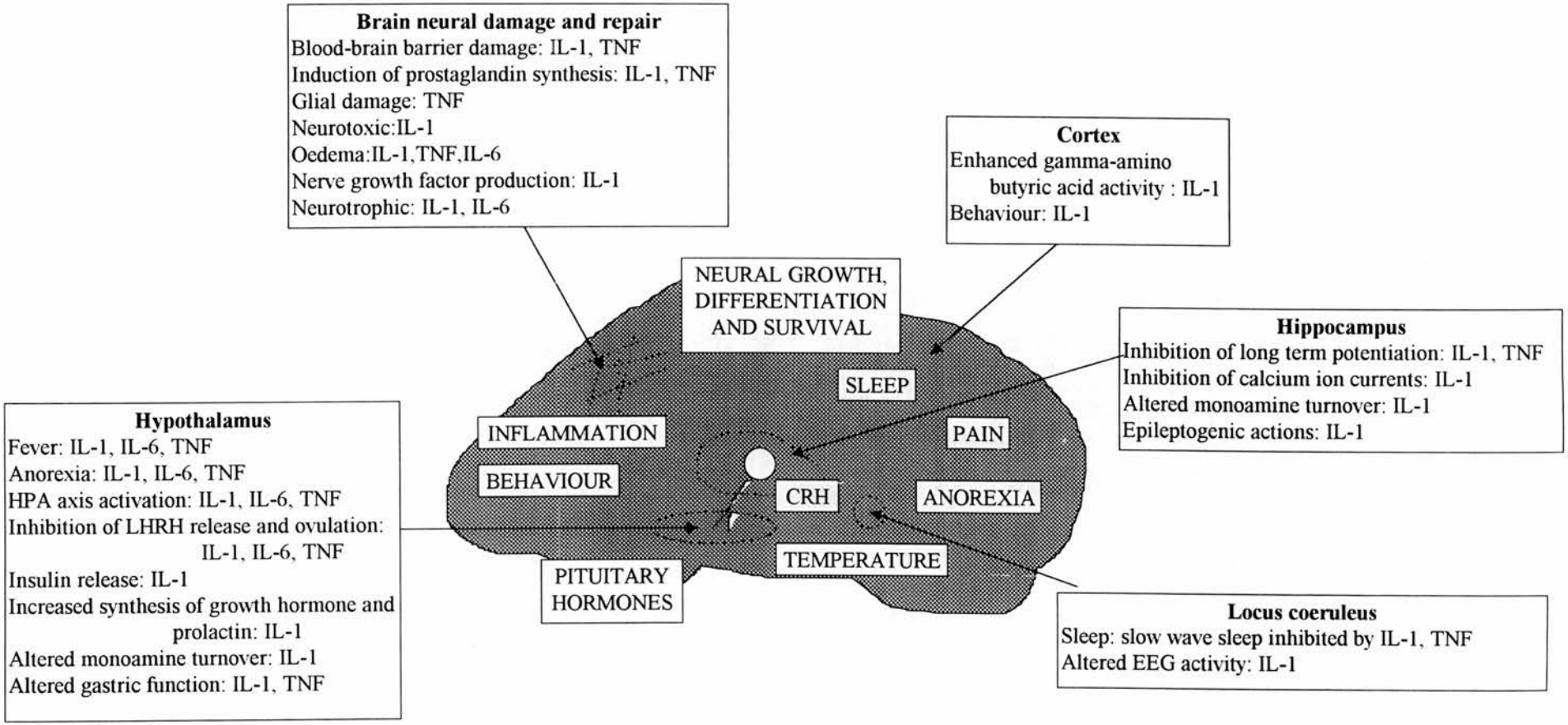
IL-1β is the main secreted form of IL-1[5] and has many diverse actions. It has a molecular weight of about 17.5kDa, and has been implicated in the pathophysiology of many disease states. including alcoholic liver disease[6], septic shock[7] and rheumatoid arthritis[8]. There is much interesting laboratory work on IL-1β in brain injury.

Woodroofe et al. showed a rise in cortical IL-1β in the 24 hours after insertion of microdialysis probes into rats' brains, thus causing penetrating injury[9]. This was the first time microdialysis had been used to measure concentrations of cytokines *in vivo;* the advantage of this technique was that it permitted repeated sampling from the same rat, which was conscious and able to move freely. In a fluid percussion trauma model in the rat, Taupin et al. showed that IL-1β production increased rapidly to a maximum at 8 hours post injury, remained increased until 18 hours, and then decreased[10]. This model of traumatic brain injury reproduces most of the neuropathological changes seen in human brain tissue after trauma. The rise in IL-1β was seen earlier than in Woodroofe's study, and the increase was more transient. This may be because by

Table 1.2 - Systemic actions of the cytokines

Cytokine	Main systemic actions
IL-1β and TNFα*	fever
	neutrophilia
	increased endothelial permeability
	alteration of iron and zinc metabolism
	decreased gastric emptying
	acute phase response
	hypotension
	tumour necrosis
	increased TNFα (IL-1β*) and IL-6 release
	upregulation of adhesion molecule expression
IL-6	induction of hepatic acute phase response
	increased endothelial permeability
	actions on B-cells and T-cells
	cell growth and differentiation
	decreased IL-1β and TNFα
	upregulation of adhesion molecules

Figure 1.1 - Cytokine actions within the brain


Brain neural damage and repair
Blood-brain barrier damage: IL-1, TNF
Induction of prostaglandin synthesis: IL-1, TNF
Glial damage: TNF
Neurotoxic:IL-1
Oedema:IL-1,TNF,IL-6
Nerve growth factor production: IL-1
Neurotrophic: IL-1, IL-6
Cortex
Enhanced gamma-amino butyric acid activity : IL-1
Behaviour: IL-1
NEURAL GROWTH, DIFFERENTIATION AND SURVIVAL
Hippocampus
Inhibition of long term potentiation: IL-1, TNF
Inhibition of calcium ion currents: IL-1
Altered monoamine turnover: IL-1
Epileptogenic actions: IL-1
SLEEP
INFLAMMATION
PAIN
BEHAVIOUR
CRH
ANOREXIA
TEMPERATURE
PITUITARY HORMONES
Hypothalamus
Fever: IL-1, IL-6, TNF
Anorexia: IL-1, IL-6, TNF
HPA axis activation: IL-1, IL-6, TNF
Inhibition of LHRH release and ovulation: IL-1, IL-6, TNF
Insulin release: IL-1
Increased synthesis of growth hormone and prolactin: IL-1
Altered monoamine turnover: IL-1
Altered gastric function: IL-1, TNF
Locus coeruleus
Sleep: slow wave sleep inhibited by IL-1, TNF
Altered EEG activity: IL-1

using the microdialysis technique, Woodroofe measured only the extracellular pool of IL-1β immediately surrounding the catheter.

Yan et al. found increased IL-1β mRNA expression in a rat model within hours of a traumatic brain injury [11]. This elegant study which incorporated many techniques of measurement including microdialysis, immunohistochemistry and in situ hybridization also showed that this increased expression was bilateral and not restricted to the injured area. Giulian et al. showed that microglia were a source of IL-1β[12], which was a potent stimulant of gliosis[13] [14]. This response was at a later stage after injury than the changes seen in Yan's study.

Other researchers showed that cerebral IL-1β was acutely increased after permanent focal ischaemia in a middle cerebral artery occlusion stroke model[15], and that cerebral IL-1β mRNA expression peaked 3 hours after onset of ischaemia[16]. Another study, which used a rat model of cerebral ischaemia, showed a peak in IL-1β mRNA expression 4 hours after injury[17].

Several laboratory studies showed the toxic effects of IL-1β. It caused hypotension in rabbits[18] (the combination of IL-1β and TNFα was synergistic), marked neuronal injury in foetal brain cell cultures[19] and potentiated the lethal effects of TNFα in mice[20]. In addition to its direct effects, IL-1β acts to stimulate production of other cytokines, including TNFα[21] and in particular IL-6, by both astrocytes and microglia[22-27].

There are fewer clinical studies which have implicated IL-1β in brain injury, possibly because it is produced mainly in the first hours after injury. Poulton et al. found increased IL-1β in the cerebral cortex after subarachnoid haemorrhage, and

suggested that this increase may be associated with the development of cerebral vasospasm[28]. This group had the good fortune to have access to human brain tissue specimens which had been removed during the surgical repair of a ruptured aneurysm. Tarkowski et al. showed that IL-1β was increased in the cerebrospinal fluid of patients who had sustained a stroke[29], and noted that the degree of increase seen was higher in those with a major stroke, however numbers of patients was relatively small.

Craig McClain and his research group from Kentucky carried out some of the first and most important early work on the cytokines in brain injury, in which patients who had sustained a traumatic brain injury were studied. Their particular interest was the relationship between cytokine production after injury and metabolic changes seen in this patient population. They found increased IL-1β in patient ventricular fluid after a traumatic brain injury[3]. Although the sample was small (n=12), the group was able to follow patients through to day 21 after injury.

The evidence available to date shows that IL-1β is produced after primary brain injury, and suggests that it may be a mediator in the secondary inflammatory response, which results in further damage to the brain in the hours and days after the injury. We shall discuss the effects of IL-1β on adhesion molecule expression later in this review.

Tumour Necrosis Factor α

TNFα has much in common with IL-1β. It too is increased in the serum in non-neurological disease, such as sepsis[30], burns[31 32], alcoholic liver disease[6] and multiple trauma[33].

Again, most of the studies in this area are experimental: in one of the most important animal studies of TNFα in traumatic brain injury Shohami et al. showed increased TNFα in contused rat brain at 1 hour post injury (weight-drop model). Concentrations peaked by 4 hours and began to fall[34]. The group also looked at tissue on the side of the brain contra-lateral to the injury. Positive results were seen at 4 hours, but concentrations were only 10% of those found in the contused hemisphere.

Similarly, in the same study described earlier for IL-1β, Taupin et al. showed that the maximal increase in TNFα in traumatized rat brain was at 3 hours, and that concentrations fell to baseline by 18 hours[10]. In a rat stroke model, Liu et al. showed that TNFα mRNA expression was induced and detected immunohistochemically in neurons by 1 hour after the onset of ischaemia[35]. These findings suggest that TNFα, together with IL-1β, is released very early after injury, and may go some way towards explaining why these two cytokines are often not detected in the serum in clinical studies.

Several investigators have studied the toxic effects of TNFα: one gave either TNFα or endotoxin to volunteers and showed that both induced a similar metabolic response including fever, stress hormone and acute phase protein release[36]. TNFα infusion in mice caused lymphopaenia, neutrophilia, shock and watery diarrhoea[37] and TNFα administered to neuronal cell cultures caused severe neuronal injury[19]. These

studies, although of interest, involve the administration of unphysiological concentrations of the cytokine and therefore the results must be interpreted with caution.

Kwang Sik Ki et al. gave intra-cisternal injections of TNFα to rats, which altered blood-brain barrier permeability and increased cerebrospinal fluid white cell count[38]. Using an argument similar to that above, this result is important, but perhaps not quite so important as those studies in which the physiological actions of the cytokines are blocked by antagonists.

There is evidence to suggest that IL-1β[21] and lipopolysaccharide (LPS, endotoxin))[39] cause increased TNFα production in the CNS, whereas IL-6 causes decreased production of TNFα by monocytes[40], suggesting a negative feedback process. TNFα itself, in a similar fashion to IL-1β, causes the release of other cytokines, particularly IL-6[22 24-27 41]. In a study by Sheron et al. Hepatitis B sufferers were treated with TNFα, which caused increases in serum IL-6 and IL-8[42].

In neurological disease, Waage et al. showed that serum TNF was increased in patients with meningococcal infection, and that the increase was more marked in those who were hypotensive[43]. Goodman et al. showed increased serum TNFα in 21 patients after traumatic brain injury; although numbers were small, increased concentrations of serum TNFα were seen in all of the patients. This is the only human study to show such large rises in serum concentrations of TNFα after traumatic brain injury. The group were unable to demonstrate any correlation between serum concentrations and outcome[44]. In 1994, Ross et al. showed that TNFα was present in the serum of 18 out of 50 patients with traumatic brain injury[1]. Each patient had only

one measurement taken, and so any changes in TNFα seen with time were unfortunately missed. However 26 had samples of CSF analysed and TNFα was found in 17 of these.

Again the evidence is strongly suggestive that TNFα is produced in the brain after injury. It may be an important factor in the propagation of the inflammatory process in the early stages after a brain injury.

Interleukin-6

Many investigators have studied IL-6 in tissue injury and disease. It is a 26kD protein which differs from IL-1β and TNFα in that it both causes and inhibits inflammation[4 40]. A particularly important function of IL-6 in brain injury is its action to increase endothelial permeability[45]. There are many review articles which cover its functions in detail[46-48]. Several studies found increased serum IL-6 in non-neurological disease, particularly in the systemic inflammatory response syndrome[30 49] where mortality was correlated with serum concentrations of IL-6. Serum IL-6 was also increased in nosocomial infection in the intensive care unit[50], after multiple trauma[33] and cardio-pulmonary bypass[51]. In many of these studies, disease severity correlated with IL-6 concentrations. There has been some debate about whether IL-6 is actually an active mediator in the inflammatory process or is simply a marker of the severity of injury[52], but the available evidence clearly implicates IL-6 in the pathophysiology of inflammatory brain injury.

Hariri et al. found increased IL-6 production by astrocytes in a model in which human astrocyte cell lines were cultured in the laboratory and subjected to a mechanical injury using fluid percussion barotrauma[53]. Injury of increasing severity

was related to IL-6 production. Similarly, Shohami et al. showed increased IL-6 in contused rat brain, which peaked 8 hours after injury[34]. Other investigators showed similar increases in IL-6 in trauma models[9-11], which usually lagged behind the production peaks of IL-1β and TNFα.

There are many clinical studies which investigate the part played by IL-6 in brain injury. Osuka et al. investigated the theory that increased serum IL-6 was related to brain tissue damage and, in an interesting study, measured IL-6 in the serum of 70 patients after neurosurgery. They found a rise which peaked 24 hours after surgery[54] and decreased back to pre-operative concentrations over the following 7 days. The magnitude of the increase was related to the type and duration of the surgery. In a similar study Heesen et al. showed that serum IL-6 was increased in 20 patients after craniotomy, peaking 24 hours post-operatively, although they did not go on to measure serum concentrations of IL-6 after 24 hours[55].

In 30 patients with a traumatic brain injury, McClain et al. found increased serum concentrations of IL-6, which were highest on admission, and highly increased ventricular fluid IL-6. The fall in serum IL-6 concentration correlated with clinical improvement, and concentrations fell more rapidly in those with a Glasgow Coma Score (GCS) greater than eight[2]. This is one of the most commonly cited papers by those who believe that IL-6 plays a major role in inflammatory brain injury. In a more recent study of 22 patients with traumatic brain injury, Kossman et al. found increased ventricular fluid concentrations of IL-6 and a relationship between IL-6 concentration and production of nerve growth factor[56].

Clinical studies in non-traumatic brain injury further implicate IL-6 as a mediator in the secondary injury process. Several investigators have studied IL-6 in

stroke patients: Tarkowski et al. found increased IL-6 in the cerebrospinal fluid of 30 stroke patients, with less pronounced serum rises[29]. This study is notable because patients were followed up for 3 months. Concentrations peaked at days 2-3, but IL-6 was still present in the cerebrospinal fluid at day 90 after the stroke. There was a significant correlation between early intrathecal production of IL-6 and the size of the brain lesion.

Beamer et al. found increased plasma concentrations of IL-6 in 50 stroke patients, with the highest concentrations in those with large infarcts[57] whilst Kim et al. studied serial plasma concentrations of IL-6 in 29 patients with non-traumatic acute brain injury and found similar rises which peaked at day 1 but persisted at day 7[58]. In subarachnoid haemorrhage, cerebrospinal fluid IL-6 was increased, peaking at day 6[59], and in another study both IL-6 and IL-8 concentrations were increased in cerebrospinal fluid[60]. An interesting study by Amado et al. found that the concentration of IL-6 in the plasma of patients rose at the time of diagnosis of brain death[61].

As we have already discussed, IL-1β and TNFα, together with many other mediators including platelet activating factor, interferon and the bradykinins[62-64], promote IL-6 production. The part played by IL-6 in the intracranial inflammatory process is complex, because it has pro- and anti-inflammatory actions, and has a negative feedback effect on the production of IL-1β and TNFα[40](Table 1.2), but it would appear to occupy a central point in the web of mediators which are involved in the inflammatory response to brain injury.

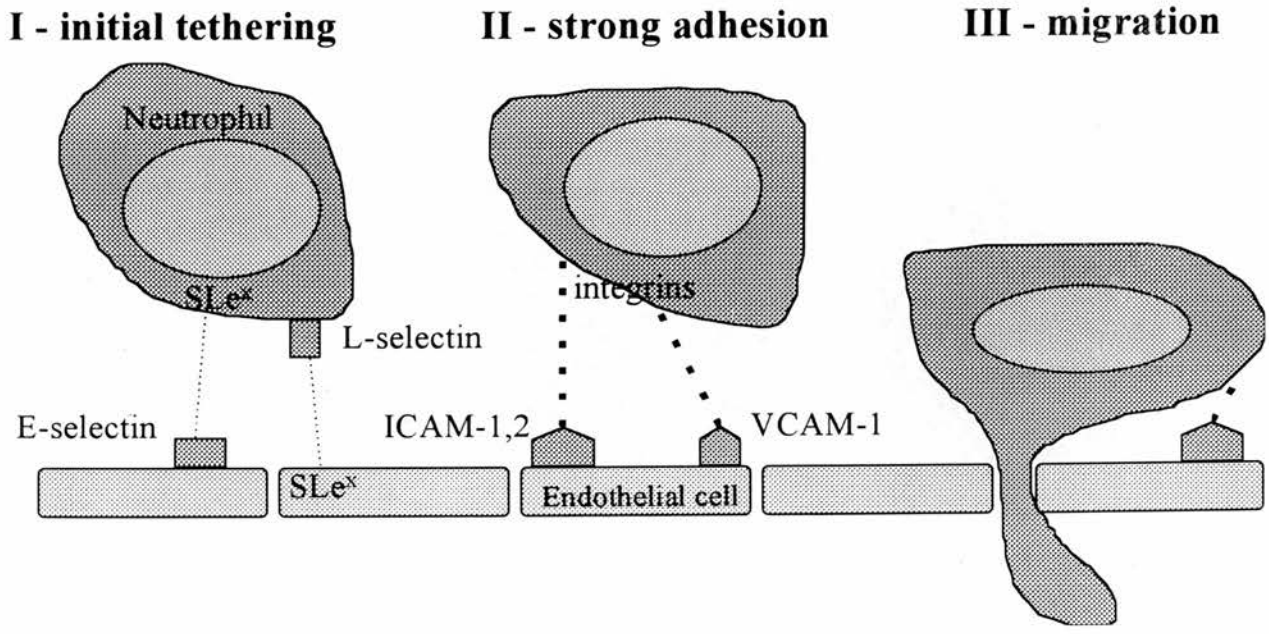
THE CYTOKINES AND THE ADHESION MOLECULES

There are three families of adhesion molecule[65]. The first includes the glycoproteins L-selectin and E-selectin, which are present on the surface of leucocytes and endothelial cells respectively. These mediate the initial tethering of leucocytes to the vessel wall by binding to endothelial receptors[66]. Deficiency of either the selectins or their ligands causes failure of the migration process and recurrent infections[67 68].

The other two families mediate strong adhesion and migration across the endothelium into tissues. The immunoglobulin "superfamily" consists of intercellular adhesion molecule (ICAM) 1 and 2 and vascular cell adhesion molecule (VCAM) 1, which are present on the endothelial surface. Their counter-receptors form the third family, the integrins (eg CD11b and CD18), which are present on the leucocyte cell surface (Figure 1.2). The blood of healthy humans contains soluble, active forms of the selectins and immunoglobulins (eg sL-selectin, sICAM-1)[69]. This review will focus on those molecules which have been most closely studied in brain injury - namely ICAM-1, E- and L-selectin and the integrins.

Before we look at the part played by the adhesion molecules in detail, we should examine the relationship between the cytokines previously discussed and control of adhesion molecule activity. Experimental work suggests that IL-1β, IL-6 and TNFα all upregulate the activity of these molecules: an early study by Bevilacqua et al. showed that treatment of cultured human vascular endothelial cells with IL-1β resulted in increased polymorphonuclear cell adhesion by acting on endothelial cells themselves. Neither pre-treatment of leucocytes with IL-1 nor the addition of IL-1 during the assay affected endothelial-leucocyte adhesion[70].

Figure 1.2 - Stages of neutrophil adhesion to, and migration across, the endothelium.



Duits et al. treated leukaemic cell lines with IL-6 and found increased surface expression of ICAM-1[71] and Hutchins et al. reported similar findings in breast cancer cell lines[72]. IL-1β, but not IL-6, increased ICAM-1 expression in rat mesangial cells[73] and IL-1β and TNFα induced expression of L-selectin on the activated endothelium of umbilical vein[66]. Other investigators showed that IL-1β increased ICAM-1 expression in fibroblasts[74] and that IL-1β and TNFα increased ICAM-1 expression in hepatocyte cell lines[75].

Hahne et al. showed how diverse the actions of a single cytokine may be when they discovered that TNFα induced expression of five different types of adhesion molecule in mouse endothelioma cells: ICAM-1, VCAM-1, E-selectin, P-selectin and one molecule not previously described[76]. This complex study is one of the few to look at the effects of cytokines on the expression of P-selectin, a member of the selectin family only expressed on vascular endothelium after tissue injury.

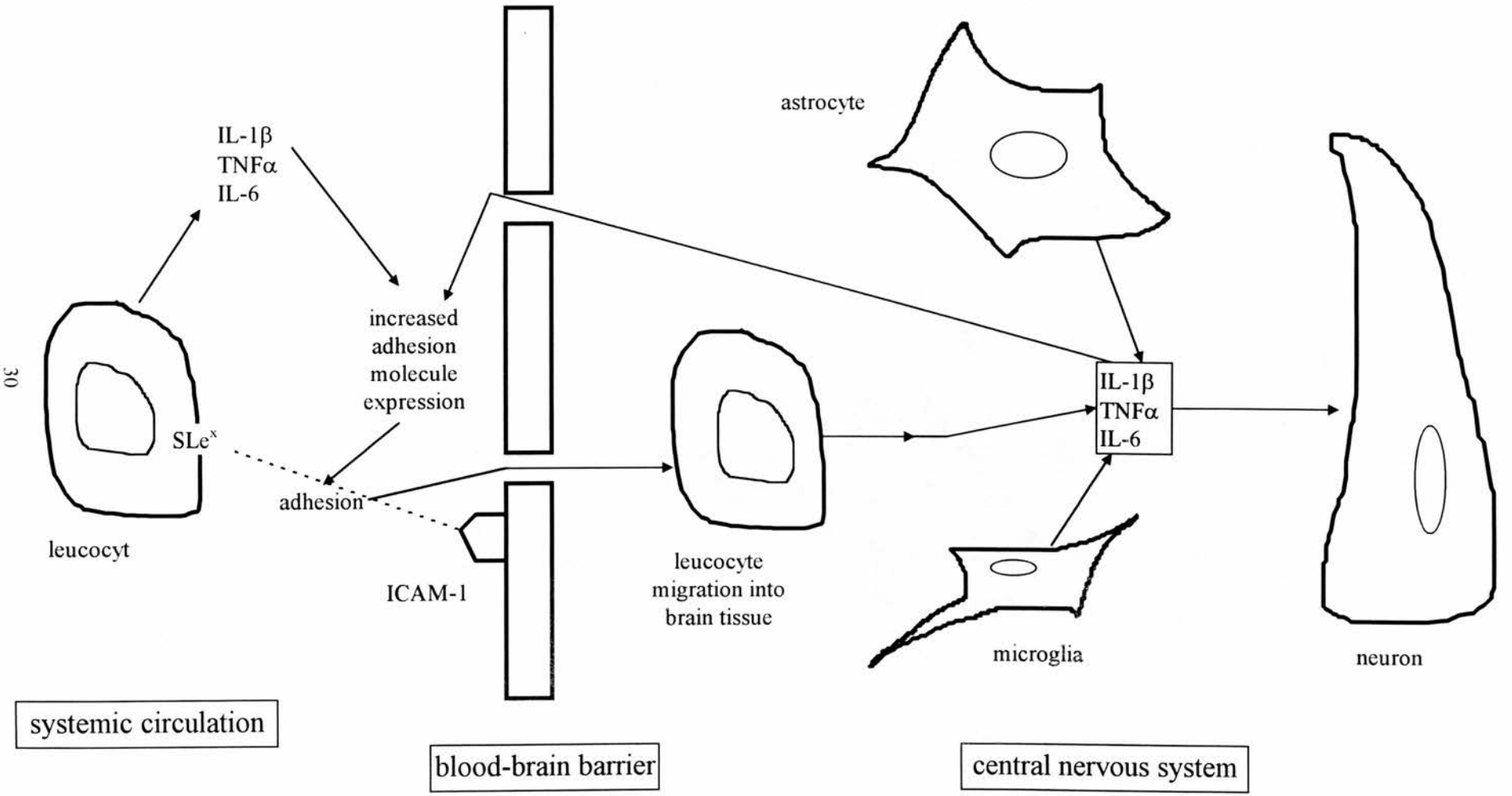
There has been little work which has studied any link between IL-6 production and adhesion molecule expression after brain injury. We therefore have to look to studies of other diseases for suggestions as to how IL-6 may be involved in adhesion molecule activation. Kukielka et al. have published considerable findings on the relationship between IL-6 and the upregulation of ICAM-1 in the pathophysiology of reperfusion injury in the myocardium[77-79]. They showed that increased IL-6 mRNA expression was seen in a canine myocardial reperfusion model, and that this was followed by an increase in ICAM-1 expression in the same segments. In a clinical study which examined 22 patients admitted to the intensive care unit with illnesses resulting from liver cirrhosis, leucocyte expression of the integrins CD11b and CD35 correlated with the concentration of IL-6 in plasma[80].

There is certainly evidence that the cytokines are involved in the regulation of adhesion molecule activity in non-neurological disease, and further experimental studies which studied brain tissue add weight to this important relationship in brain injury.

Zielasek et al. found that TNFα and interferon-gamma caused an increase in ICAM-1 expression over normal baseline expression in isolated rat microglia[81]. DoreDuffy et al. studied the effects of TNFα on rat CNS endothelium *in vitro* and found increased expression of VCAM-1 and E-selectin; this study was important because intact endothelium was studied rather than just cultured endothelial cells[82]. In a similar study by Wong and DoroviniZis, IL-1β ,TNFα, interferon-gamma and LPS all increased ICAM-1 expression in primary cultures of human brain microvessel endothelial cells[83]. In a study in which the investigators had early access to human brain tissue post-mortem, Rieckmann et al. found that ICAM-1 was mainly expressed on endothelial cells of small vessels, and showed that expression of ICAM-1 was increased in tissue sections after stimulation with TNFα and LPS[84].

These studies suggest that changes in local concentrations of cytokines seen after acute brain injury may affect the activity of adhesion molecules in the CNS, which may then increase polymorphonuclear cell infiltration and cause further cellular damage (Figure 1.3). We shall now go on to look at the adhesion molecules, and the part they play in brain injury, more closely.

Figure 1.3 - Intracranial cytokine and adhesion molecule interactions


IL-1β
TNFα
IL-6
astrocyte
increased
adhesion
molecule
expression
SLe^x
adhesion
leucocyt
ICAM-1
leucocyte
migration into
brain tissue
IL-1β
TNFα
IL-6
microglia
neuron
systemic circulation
blood-brain barrier
central nervous system

THE ADHESION MOLECULES

Clinical research into the role of the adhesion molecules in disease is still in its infancy, particularly in the area of acute brain injury, but many studies suggest that manipulation of the effects of these molecules may prove to be a powerful tool in the treatment of inflammatory disease. Detailed reviews of the adhesion molecules can provide additional background information[65 85 86]. The most extensively studied molecule is ICAM-1, a member of the immunoglobulin "superfamily". This molecule is expressed on the vascular endothelium and, as has already been stated, facilitates strong adhesion and migration of white cells from vessels into tissue. There is also evidence of its role in the intra-parenchymal binding of white cells to tissue cells such as astrocytes in the CNS[87 88].

The soluble forms of the adhesion molecules (eg sICAM-1) are those which can be measured by assay in the serum or plasma of normal individuals[69], but the part they play in the physiological response to injury is unclear. Increased serum concentrations of the soluble endothelial molecules (sICAM-1, sE-selectin) may result from damage to the endothelium as a result of systemic inflammatory processes. Alternatively, these same processes may cause an "activation" of the endothelium, with upregulation of production and shedding of these molecules into the blood[89 90].

Again laboratory studies add weight to the evidence provided by the small number of clinical studies on this subject in brain injury. Hallenbeck et al. and Garcia et al. showed that polymorphonuclear cells accumulate in damaged brain tissue[91 92]. The former group published their work in 1986, having studied a canine model of brain ischaemia in which granulocyte accumulation was found in the injured hemisphere

within 60 minutes of injury. The latter group used a relatively large sample (n=96) of rats in their study in which they induced ischaemia by ligating the middle cerebral artery. They also found evidence of early infiltration of leucocytes - polymorphs were detected in the microvessels of the injured hemisphere as early as 30 minutes after the onset of ischaemia. Schoettle et al. found a significant correlation between degree of polymorphonuclear cell accumulation after brain trauma and extent of cerebral oedema in rat fluid percussion model[93]. This group was the first to show a relationship between leucocyte infiltration and oedema after brain injury.

There is little laboratory work on the adhesion molecules in traumatic brain injury; most studies examine adhesion molecules in stroke models, although this work is certainly relevant to all forms of cerebral ischaemia. Wang et al. showed, in a study using a rat stroke model, and including both Northern blot and sensitive RT-PCR analysis methods, an increase in cortical E-selectin expression by 6 hours post-injury, which peaked at 12 hours and returned to basal concentrations by day 5. ICAM-1 expression was also increased by 3 hours post-injury and remained increased for 5 days.[94 95].

Clark et al. studied CNS reperfusion injury in rats, with a temporary middle cerebral artery occlusion model. This important study showed an increase in cortical ICAM-1 expression from 1 hour post-injury, which persisted for 72 hours. This correlated with a moderate infiltration of polymorphonuclear cells in the areas of ICAM-1 expression[96]. Haring et al. studied cerebral reperfusion injury in primates, using a temporary middle cerebral artery occlusion model, and found an upregulation of E-selectin in the ischaemic microvessels after injury[97].

In one study looking at a model of subarachnoid haemorrhage in rats, Handa et al. showed an increased expression of ICAM-1 on cerebrovascular endothelial cells[98]. Soriano et al. recently showed that ICAM-1 deficient mice were less susceptible to cerebral ischaemia / reperfusion injury[99]. This study is particularly interesting, as the fact that a genetically engineered deficiency of ICAM-1 reduces susceptibility to the effects of reperfusion injury provides powerful evidence that ICAM-1 is a factor in the pathophysiology of reperfusion injury.

Many of the clinical studies on the subject of adhesion molecules carried out to date have been in the systemic inflammatory response syndrome patient population. Cowley et al. found increased concentrations of sICAM-1, sVCAM-1 and sE-selectin in sepsis, particularly if there was organ dysfunction. A high sE-selectin concentration correlated closely with multiple organ dysfunction and death[100]. In other studies of septic patients, sICAM-1[89] and sE-selectin[101] were increased in sepsis (concentrations of sICAM-1 were significantly higher after 24 hours in non-survivors than survivors).

Patients with multiple trauma have also been studied. Boldt et al. found that sICAM-1 was increased in sepsis and after trauma, but the rise was greater in the sepsis group. sE-selectin and sVCAM-1 were increased in sepsis but not in trauma[102]. Law et al. showed that a rise in serum SICAM-1 after multiple trauma correlated with the severity of subsequent multiple organ dysfunction[90].

sL-selectin is increased in cerebral malaria[103] and in a study which examined human brain tissue post mortem, ICAM-1 expression was increased in the cerebral vessels of patients with the diagnoses of multiple sclerosis, viral encephalitis and cerebral infarct[104]. Rieckmann et al. found a high concentration of sICAM-1 in the cerebrospinal fluid of patients with bacterial meningitis and other inflammatory

neurological diseases such as multiple sclerosis and polyradiculitis. Concentrations of sICAM-1 correlated with measures of blood-brain barrier dysfunction[105]. sICAM-1 was found in the cerebrospinal fluid of patients with non-inflammatory neurological diseases such as tumours and degenerative disease in only 3 out of 50 cases (6%). Jander et al. also showed high cerebrospinal fluid and serum concentrations of sICAM-1 in bacterial meningitis[106].

Kim et al. studied integrin expression on leucocytes from patients with the diagnosis of stroke or transient ischaemic attack and, although patient numbers were small (n=16), found increased expression of CD11a and CD18 within 72 hours after the onset of ischaemia[107]. Lindsberg et al. studied brain sections of patients who had died from stroke disease. This is one of the few ways in which human tissue can be studied in detail. 11 subjects who died between 15 hours and 18 days after sustaining a stroke were studied. The group showed a striking upregulation of endothelial ICAM-1 in the cerebral microvessels[108].

There are no published studies to date which investigate serial changes in adhesion molecules in traumatic brain injury or spontaneous subarachnoid haemorrhage, but Keskil reported that in a group of 153 patients with a traumatic brain injury there was a good correlation between injury grading and admission white cell count[109].

In a recent stroke study, Fassbender et al. found increased sICAM-1 and reduced sL-selectin in patients with significant risk factors for stroke, and in addition found increased sICAM-1, reduced sL-selectin, increased sE-selectin and sVCAM-1 in a group who had sustained a stroke[110]. This fall in sL-selectin may be explained by the notion that activated endothelial counter receptors "mop up" the pool of sL-selectin in

serum. A study by Donnelly et al. supported this theory. They found reduced sL-selectin in the serum of a sub-group of patients who progressed to develop the acute respiratory distress syndrome from a sample judged to be "at risk" on recruitment to the study[111]. Blann et al. proposed a similar theory to explain their findings of a reduced concentration of sL-selectin in patients with systemic sclerosis and vasculitis. They also showed an inverse relationship between serum sL-selectin and disease severity[112].

The rather limited evidence available suggests that acute brain injury may result in an increased expression of leucocyte adhesion molecules on the cerebrovascular endothelium, and in brain tissue itself, with expression mediated in part by the cytokines. The final step in the investigative pathway is to examine the effects of cytokine and adhesion molecule antagonists on the process, and to examine whether these compounds may offer a potential benefit after brain injury.

ANTICYTOKINE AND ANTIADHESION MOLECULE THERAPY

There are several questions to be answered in the final part of this review. What effects do anticytokine therapies have on cerebral tissues and expression of adhesion molecules after acute brain injury? Do antibodies to adhesion molecules reduce secondary damage after a cerebral insult? What effects do these potential therapies have in injuries to other organ systems?

Let us consider the last question first. Few physicians in the specialities of anaesthesia or intensive care will be unaware of the interest in anticytokine therapy in

the management of the systemic inflammatory response syndrome in recent years, and the reporting of findings of several multicentre trials. Fisher et al. published the findings of two trials in 1993 and 1994. The first looked at treatment of the systemic inflammatory response syndrome with anti-TNFα antibody and showed no apparent benefit[113]. The second examined the effects of an IL-1β receptor antagonist (IL-1βra) and showed no increase in survival in the treated group[114].

In 1996, Cohen et al. reported the findings of the "Intersept" trial in which 564 patients with the systemic inflammatory response syndrome were randomised to receive TNFα antibody therapy or placebo[115]. Again there was no significant difference in mortality, but the treated group showed a more rapid reversal of shock and a longer time until first organ failure.

These findings do not encourage optimism in those who hope that anticytokine therapy may aid acute brain injury management, but there has been some criticism of the design of these studies and it may be that significant treatment effects were missed. We must remember that the cytokines function as natural communication molecules in host defence, and so antagonism may also have detrimental effects.

In experimental anticytokine therapy in the laboratory, however, findings are more encouraging. Giulian et al. showed that in glia-neuron co-cultures, activated microglia produced soluble factors which increased the population of microglial cells and reduced numbers of neurons. IL-1βra blocked this astroglial proliferative effect[116]. Studies of this type, in which antagonists to a cytokine or adhesion molecule block a particular pathophysiological process, either *in vitro* or *in vivo*, offer some of the most powerful evidence available for the involvement of these molecules in brain injury.

Kwang Sik Ki et al. showed that the increase in blood-brain barrier permeability and cerebrospinal fluid white cell count caused by the administration of intra-cisternal TNFα was abolished by treatment with a TNFα antibody[38]. Chao et al. gave IL-1β and TNFα together to foetal brain cell cultures. Administration of both nitric oxide inhibitors and NMDA receptor antagonists reduced the resultant marked neuronal injury[19]. Although both are interesting studies, the fact that cytokines have been artificially introduced to the experimental environment in doses likely to be above those seen physiologically weakens their value.

Two studies examined the effects of anti-cytokine therapy on cerebral oedema: Yamasaki et al. showed that in a rat middle cerebral artery occlusion model, the administration of an IL-1β blocker reduced cerebral oedema[117]; more recently Shohami et al. showed that after traumatic brain injury in the rat, TNFα antibody therapy caused a reduction of cerebral oedema at 24hrs post-injury, and facilitated recovery of motor function[118]. Both studies offer a tantalizing glimpse of the therapeutic potential that some investigators believe these agents may offer.

Toulmond et al. gave IL-1ra to rats who had sustained a traumatic brain injury. In the group where treatment was given immediately after the injury, a reduction in lesion size, assessed histologically, of 44% was found. Even if treatment was delayed by 4 hours, a 28% reduction in lesion size was seen[119]. Shibayama et al. showed that treatment of mice who had sustained a penetrating stab wound to the brain with antibody to IL-1β resulted in a significant reduction of ICAM-1 positive glia at 24 and 48 hrs post injury[88] and Rothlein et al. showed that cytokine specific anti-sera inhibited induction of ICAM-1 in adenocarcinoma cell lines by IL-1β and TNFα[120]. These

findings would suggest that anticytokine therapy may be capable of reducing cerebral tissue damage and adhesion molecule expression after brain injury.

Finally, let us consider the therapeutic potential of blocking the actions of adhesion molecules themselves. This is theoretically a more attractive option than attempting to block the diverse actions of the cytokines, with their complex inter-relationships. Clark and Zivin have recently published a detailed review on this subject in stroke[121], as have Hartl et al.[122] and Chopp and Zhang[123], but the subject has received little attention by investigators in traumatic brain injury research.

Two groups have made substantial progress in this area using models of CNS ischaemia - Chopp and Zhang's group from Detroit and Clark's group from Oregon. They have published several interesting studies which show the potential of anti-adhesion molecule therapy in brain injury. In 1991, Clark's group published two good papers which looked at the effects of two different anti-adhesion molecule antibodies in two models of CNS ischaemia[124 125] - the first a reversible model of spinal cord ischaemia, the second an irreversible brain ischaemia model. It showed that antibody to the integrin CD18 and to the immunoglobulin ICAM-1 produced a significant reduction in neurological deficits in the former model but not the latter - findings which implicate leucocytes in CNS reperfusion injury.

The Detroit group has published findings from several well-planned studies in rat stoke models since 1994; it showed that anti-ICAM-1 antibody reduced infarct size and polymorphonuclear cell infiltrate in a temporary middle cerebral artery occlusion model in rats[126]; in a similar model, anti-CD11b antibody decreased the volume of the ischaemic lesion, but this was not so in a permanent middle cerebral artery occlusion model[127]; antibody to the Mac-1 integrin resulted in decreased weight loss,

neurological dysfunction, polymorphonuclear cell infiltration and infarct size in a temporary middle cerebral artery occlusion model[128], and the group showed that anti-ICAM-1 antibody decreased infarct size and weight loss in a temporary but not permanent middle cerebral artery occlusion model[129].

In more recent work it has looked at the effect of giving antibodies later than at the onset of ischaemia - which is much more relevant to the clinical scenario. It showed that treatment with anti-CD11b and anti CD18 antibodies 2-4 hours after the onset of ischaemia resulted in reduced infarct size and decreased myeloperoxidase activity in a temporary middle cerebral artery occlusion model[130] and also showed that treatment with anti-ICAM-1 and anti-integrin antibodies at time of reperfusion reduced infarct size and the number of apoptotic cells seen after temporary middle cerebral artery occlusion[131].

Other groups have reported similar benefits: Matsuo et al. showed a reduction in cerebral oedema, polymorphonuclear cell infiltrate and infarct size after administration of anti-ICAM-1 and anti-integrin antibody, both at 15 minutes pre-ischaemia and at reperfusion, in a rat stroke model[132]. In a spinal cord ischaemia model in rabbits, Lindsberg et al found that administration of an anti-integrin antibody reduced motor deficit and blood-brain barrier dysfunction[133].

Other antagonists may have a part to play in future anti-adhesion therapy: Nelson et al. showed that heparin oligosaccharides bind to L- and P-selectin and thus inhibit their binding to other counter-receptors[134]; Cecconi et al. showed that inositol polyanions reduced binding of L- and P-selectin in vitro[135].

The available evidence therefore suggests that administration of agents which antagonise the harmful effects of neutrophil adhesion and migration into tissue may be

beneficial, although again we must be aware that adhesion and migration of leucocytes are natural responses of the host to injury, and that antagonism of these actions may reduce the positive effects of the inflammatory response.

CONCLUSIONS

Research to date strongly implicates cytokines such as IL-1β, IL-6 and TNFα, together with the leucocyte adhesion molecules, in the pathophysiological response to primary brain injury. The cascades or webs of mediators released after primary injury cause secondary brain injury which may be severe. Further work in the areas of anticytokine and, in particular, anti-adhesion molecule therapy may result in the development of novel therapies which alleviate the devastating sequelae of acute injury to the brain.

I have reviewed the parts played by the cytokines and adhesion molecules in the pathophysiology of acute brain injury. It is clear from this review that further clinical research is necessary to investigate the effects of the intracranial inflammatory process in patients rather than just in the laboratory.

Chapter 2 describes a study carried out in the intensive care unit which examines serial changes in cytokines and adhesion molecules in patients who have sustained a brain injury, and relates these to each other and neurological outcome.

CHAPTER 2

SERUM MARKERS OF CEREBRAL DAMAGE AND INFLAMMATION AFTER ACUTE BRAIN INJURY

INTRODUCTION

As I have discussed in Chapter 1, there is much evidence to suggest that both the cytokines and the adhesion molecules play a pivotal part in the pathophysiology of the inflammatory process after both traumatic brain injury and spontaneous subarachnoid haemorrhage. There have, however, been no clinical studies published to date which look at serial cytokine and adhesion molecule concentrations in either cerebrospinal fluid or arterial and jugular venous serum after acute brain injury and relate these to neurological outcome.

There has also been interest in the measurement of neuron specific enolase, an isoform of the glycolytic enzyme enolase, which is found in neurons and neuro-endocrine cells, in the serum and cerebrospinal fluid after brain injury[136-138]. Some investigators have claimed that the concentration of this substance in serum or cerebrospinal fluid is a good quantitative marker of neuronal damage. Others have suggested that measurement of serum or cerebrospinal fluid concentrations of protein S-100, a protein present predominantly in astroglial cells, after brain injury is a suitable quantitative marker of injury severity[139-142].

With the increased use of intraparenchymal solid state methods for monitoring intra-cranial pressure, access to cerebrospinal fluid for analysis is often not possible, and where it is, sampling from catheters may result in ventriculitis[143]. Placement of a fibre-optic catheter in the jugular bulb to monitor jugular venous haemoglobin oxygen saturation is now standard practice within our intensive care unit in patients with acute brain injury, giving ready access to blood draining from the brain. We investigated the hypothesis that systemic concentrations of the cytokines IL-1β, IL-6, IL-8 and TNFα and the adhesion molecules sICAM-1 and sL-selectin would be raised after acute brain

injury, and that increased intracranial production of these molecules would result in a demonstrable transcranial cytokine gradient, with jugular venous concentrations higher than arterial. We also investigated whether there was a relationship between serum concentrations of these molecules and severity of injury as measured by Glasgow Coma Score and Injury Severity Score, type of injury, protein markers of injury (S-100 and NSE), the presence of extracranial injuries and patient outcome at 6 months after injury.

METHODS

Local Ethics Committee approval for the study was obtained. A series of 32 sequential patients who had sustained an acute brain injury (traumatic brain injury or spontaneous subarachnoid haemorrhage) requiring intensive care were recruited to the study. Informed consent was not obtained as all patients were comatose and therefore were unable to give consent. Details of the study were discussed with relatives. Data of patient characteristics, consisting of sex, age, Glasgow Coma Score (GCS) after non-surgical resuscitation and Injury Severity Score were recorded on admission to the intensive care unit (Tables 2.1, 2.2 and 2.3). A single consultant neuroradiologist classified the brain injury by type from the CT scan. Glasgow Outcome Scores (GOS) at 6 months after injury were obtained from information supplied by the patients' general practitioner. The Glasgow Outcome Scores refer to the following: 1 - dead, 2 - vegetative, 3 - severely disabled, 4 - moderate recovery, 5 - good recovery[144].

Table 2.1 - Characteristics of traumatic brain injury patient group.

Number	Sex	Age	GCS	Type of Injury	GOS
1	M	22	8	EDH	5
2	M	48	5	ASDH	1
3	M	35	8	EDH	5
4	M	40	5	DI	1
5	F	24	8	Vault fracture	U/K
6	M	17	7	HI, hypoxia	4
7	F	49	7	ASDH, DI	4
8	M	21	4	EDH	2
9	F	48	10	Cont + infarct	3
10	M	35	6	HI, hypoxia	4
11	M	19	3	DI	1
12	M	45	3	ASDH	1
13	M	54	3	BOS fracture, tSAH	1
14	M	69	13	Cont + tSAH	1
15	M	19	13	HI	4
16	F	33	11	ASDH	5
17	M	24	6	HI	4
18	M	18	6	Cont	5
19	M	37	7	EDH	3
20	M	17	4	Cont	5
21	M	55	9	HI	5
22	M	18	5	DI + Cont	4

GCS= Glasgow Coma Score
GOS= Glagow Outcome Score
EDH= Extradural haematoma
ASDH= Acute subdural haematoma
DI= Diffuse Injury
Cont= Contusions
tSAH= Traumatic subarachnoid haemorrhage
BOS= Base of skull
HI= History of TBI, normal CT scan

Table 2.2 - Characteristics of subarachnoid haemorrhage patient group.

Number	Sex	Age	GCS	GOS
1	M	32	5	3
2	F	51	7	4
3	M	65	6	3
4	F	53	5	3
5	M	38	11	1
6	F	21	7	4
7	M	52	8	4
8	F	55	10	5
9	M	54	3	1
10	M	60	7	1

GCS= Glasgow Coma Score GOS= Glasgow Outcome Score

Table 2.3 - Summary of patient characteristics data

	TBI (n=22)	SAH (n=10)
Sex: Male/Female	18 / 4	6 / 4
Age: Range (yrs)	17 - 69	21 - 65
Median (yrs)	35	52*
GCS: Range	3 - 13	3 - 11
Median	6	7
GOS: Range	1 - 5	1 - 5
Median	4	3

*= significant difference (p=0.028, t-test)

A dual lumen Edslab 4-French gauge oximetry catheter (Baxter Healthcare Corporation, Irvine, CA, USA) was placed in the jugular bulb on the dominant side of cerebral venous drainage, as previously described[145 146]. Satisfactory positioning was ensured by lateral x-ray of the cervical spine (catheter tip seen cephalad to the upper border of C2 vertebral body), and the correct catheter distance marking (eg 15cm) showing at the valve of the sheath was written in the notes for future reference. An intra-arterial catheter was placed (if not already *in situ*), usually in the radial artery as is our standard practice.

Paired arterial and jugular venous blood samples were taken at designated times after brain injury: on admission (median time after injury 8h 30 min, range 2h - 14h), at 24 hours, 48 hours and 96 hours. Time of brain injury in the subarachnoid haemorrhage group was noted as the time of sudden deterioration in Glasgow Coma Score by 3 points or more. Patients who did not exhibit a sudden neurological deterioration were not included. The diagnostic criterion for subarachnoid haemorrhage was the presence of subarachnoid blood on the CT scan. Satisfactory positioning of the jugular bulb catheter was ensured before each sample was withdrawn by checking that the correct distance marker of the catheter was showing at the sheath valve. No patient underwent craniotomy or siting of an intracranial pressure monitoring device during the period of observation. Samples were allowed to clot at room temperature, spun down in a centrifuge at 4000rpm for 10 minutes and the supernatant removed and frozen immediately at -25 degrees Celsius.

Analysis of serum for IL-1β, IL-6, IL-8, TNFα, sICAM-1 and sL-selectin was performed by enzyme linked immunosorbent assay (ELISA) (Quantikine™ and Parameter™ Assays, R&D Systems) according to the manufacturer's instructions. Inter- and intra-assay coefficients of variation for each assay are shown in Table 2.4.

Table 2.4 - Performance characteristics of each assay. To assess intra-assay precision, three samples of known concentration were assayed twenty times on one plate. To assess inter-assay precision, three samples of known concentration were assayed in twenty separate assays.

	Intra-assay coefficient of variation (%)			Inter-assay coefficient of variation (%)		
Sample	1	2	3	1	2	3
IL-1β	3.4	4.4	2.8	8.4	4.2	4.1
IL-6	4.3	1.7	2.1	6.3	3.3	7.2
IL-8	3.9	2.4	3.3	12.2	9.1	7.3
TNFα	5.2	4.2	4.6	7.4	4.6	5.4
ICAM-1	4.8	4.8	3.3	10.1	7.4	6.0
L-selectin	2.6	4.7	3.1	7.6	5.9	6.3

Analysis of serum for NSE and S-100 was by radioimmunoassay (RIA) (Sangtec Medical). All ELISAs were carried out by a single operator using the same equipment and procedures throughout, as were all RIAs. Repeat freeze-thaw cycles for serum samples were avoided by separating the serum from each patient into multiple tubes before freezing. Results for each assay were validated by the inclusion of control sera supplied by the manufacturer.

A total of 138 samples (69 pairs) were analysed in duplicate for IL-1β, IL-8 and TNFα, with an additional 80 samples (40 pairs) for IL-6, sICAM-1 and sL-selectin, and results averaged to give the final concentration. Analysis for NSE and S-100 was carried out on the serum of patients with traumatic brain injury only. Data are incomplete because of patient death, discharge from the intensive care unit or values lying outwith ELISA quantification limits. For the traumatic brain injury group, at each time point n=20, 21, 19 and 11 respectively. For the subarachnoid haemorrhage group, n=10,10, 8 and 6 respectively. Samples which contained a concentration higher than the highest standard supplied with the kit were diluted and the assay was repeated. Sera from eight healthy volunteers were analysed for use as controls for the cytokine assays, and from twelve volunteers for the adhesion molecule, S-100 and NSE assays. For the ELISAs, standard curves and concentrations were calculated by a personal computer interfaced to a micro-plate reader using dedicated software (Bio-Rad Laboratories). For the RIAs standard curves and concentrations were calculated by a personal computer interfaced to a gamma-counter.

Statistical testing was by means of SPSS Base 7.0 for Windows. P values quoted are from Wilcoxon rank sum tests, Student's t-tests, one or two way analysis of variance (ANOVA) and linear or logistic regression analysis. Both parametric and non-parametric statistical tests are used in this chapter as analysis of the cytokine data was

carried out at an earlier stage than that of the remainder of the chapter. Statistical advice at that stage was that as there were some outlying data points, non-parametric analysis should be used for that data. Advice regarding later analyses was that parametric methods were appropriate.

RESULTS

Comparing the traumatic brain injury and subarachnoid haemorrhage groups, patients in the latter group were older ($p=0.028$), but there was no difference in Glasgow Coma Score ($p=0.979$).

The Cytokines

IL-1β, TNFα and IL-8 - IL-1β was undetectable (<3.9pg/ml) in 119 of the 138 samples (86%) assayed. The highest concentration in the remaining 19 (14%) was 7.0pg/ml. TNFα was undetectable (<15.6pg/ml) in 122 samples (88%). The highest concentration in the remaining 16 (12%) was 31pg/ml. IL-8 was detected in 72 samples (52%) (lower limit of detection 94pg/ml), but only 10 (7%) showed concentrations >300pg/ml. There was no difference between jugular venous and arterial concentrations of IL-1β, IL-8 or TNFα in those patients with detectable concentrations. Control samples all showed undetectable concentrations.

IL-6 - IL-6 was detected in all 218 samples. Figures 2.1 and 2.2 show the distribution of concentrations of IL-6 in the jugular venous and in the arterial samples. In the controls, concentrations were below detectable limits (<3.1pg/ml) in 4 samples. The highest concentration in the remaining 4 control samples was 4.7pg/ml. In 4 pairs of

Figure 2.1 - Boxplots comparing median (solid line), interquartile range (shaded area), outliers (o) and extremes (*) for concentrations of IL-6 in arterial and jugular venous samples in patients with traumatic brain injury ($p<0.001$).

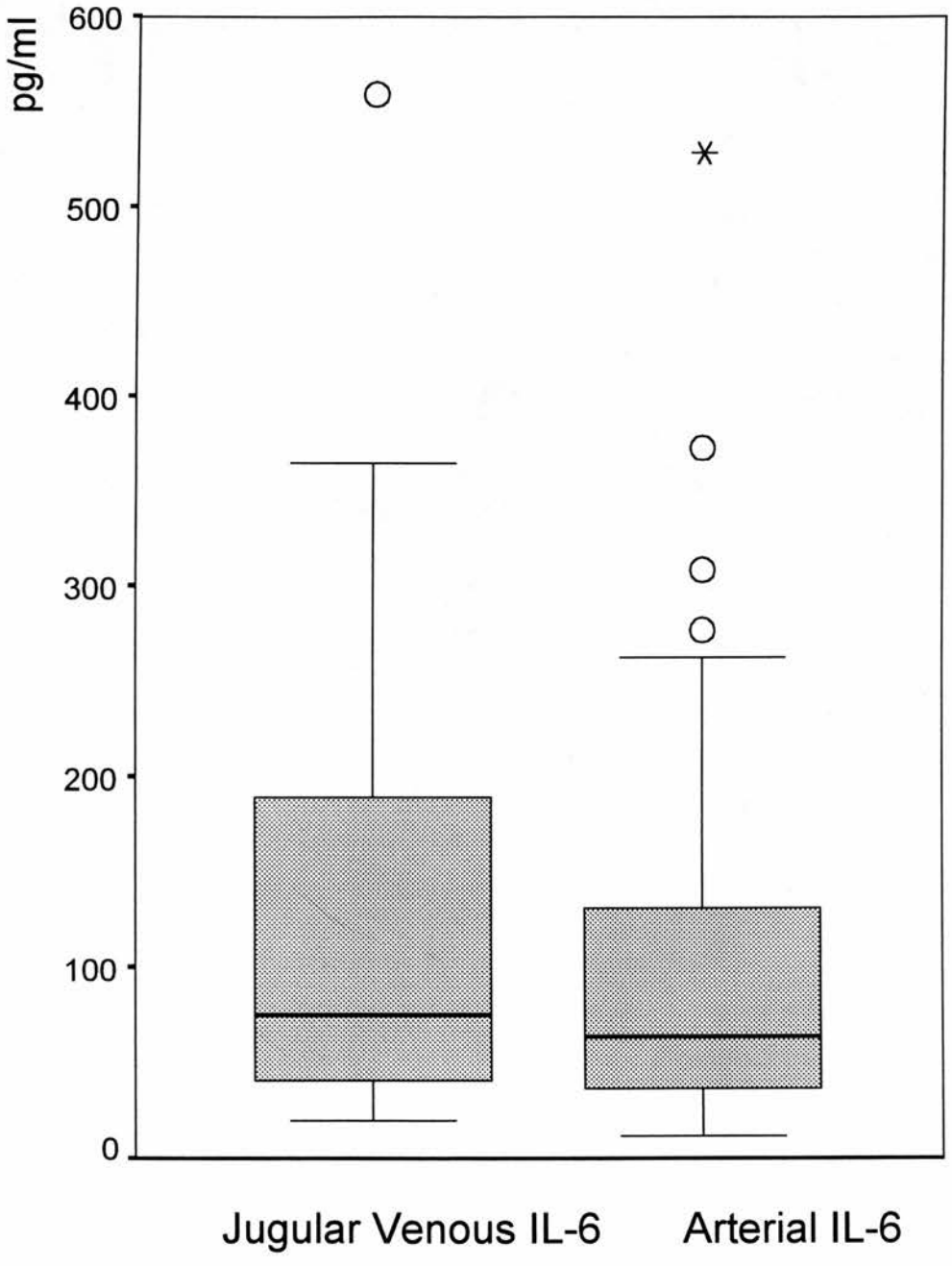
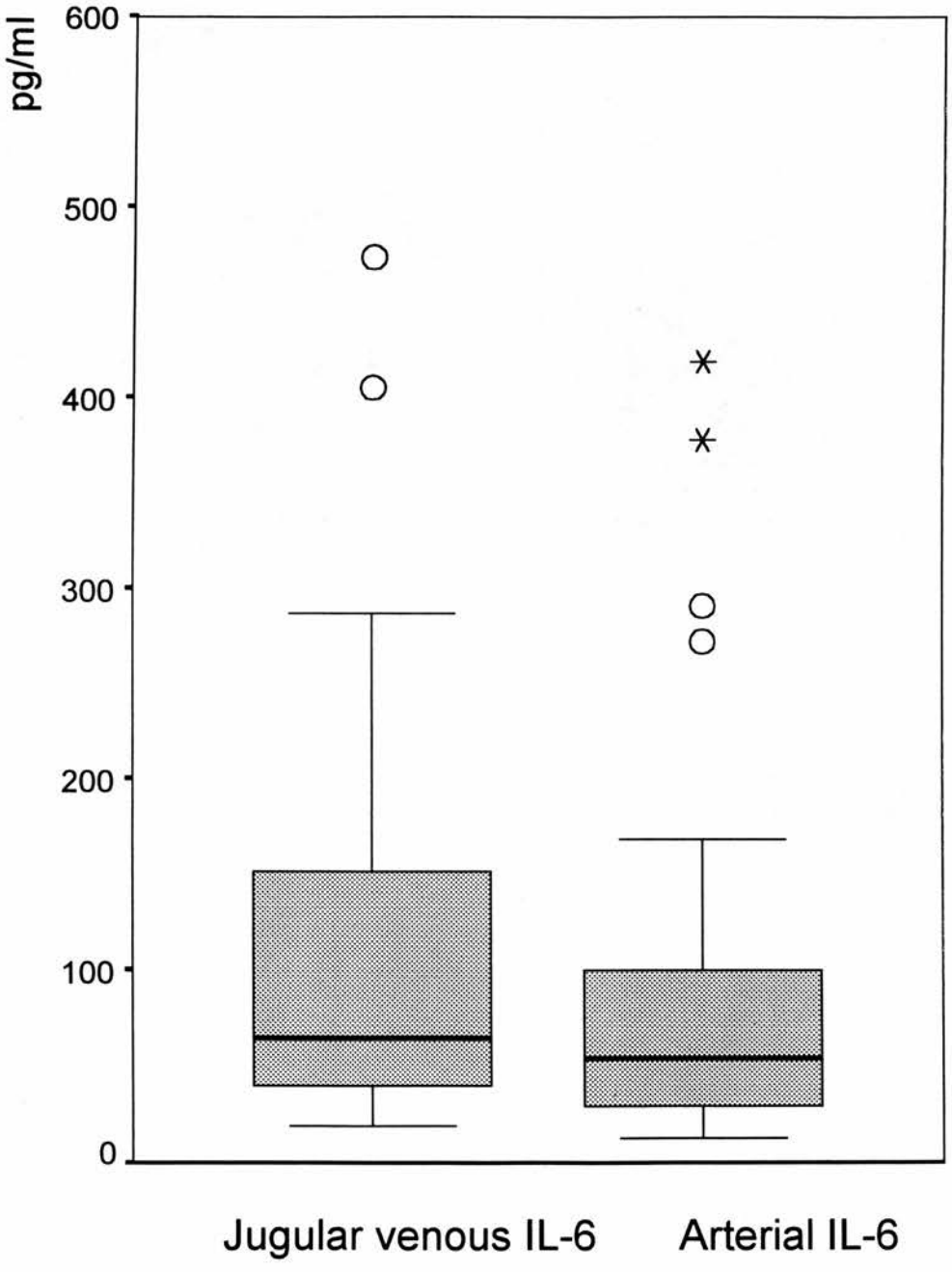


Figure 2.2 - Boxplots comparing median, interquartile range, outliers (o) and extremes (*) for concentrations of IL-6 in arterial and jugular venous samples in patients with spontaneous subarachnoid haemorrhage ($p<0.001$).



patient samples (3.7%), both samples showed concentrations >1500pg/ml (the upper limit of quantification), and were excluded from statistical analysis. In 82 (78.1%) of the remaining 105 pairs, jugular venous concentration was higher than arterial. In the traumatic brain injury group, median jugular venous concentration was 74.8pg/ml (range 19.9 to 539.8); median arterial concentration was 63.5pg/ml (range 11.4 to 518.4) . Median difference was 6.7pg/ml (range -40.8 to 244.4; $p<0.001$). In the subarachnoid haemorrhage group, median jugular venous concentration was 64.6pg/ml (range 19.1 to 455.4); median arterial concentration was 54.1pg/ml (range 12.2 to 407.4). Median difference was 11.3pg/ml (range -32 to 135.2; $p<0.001$).

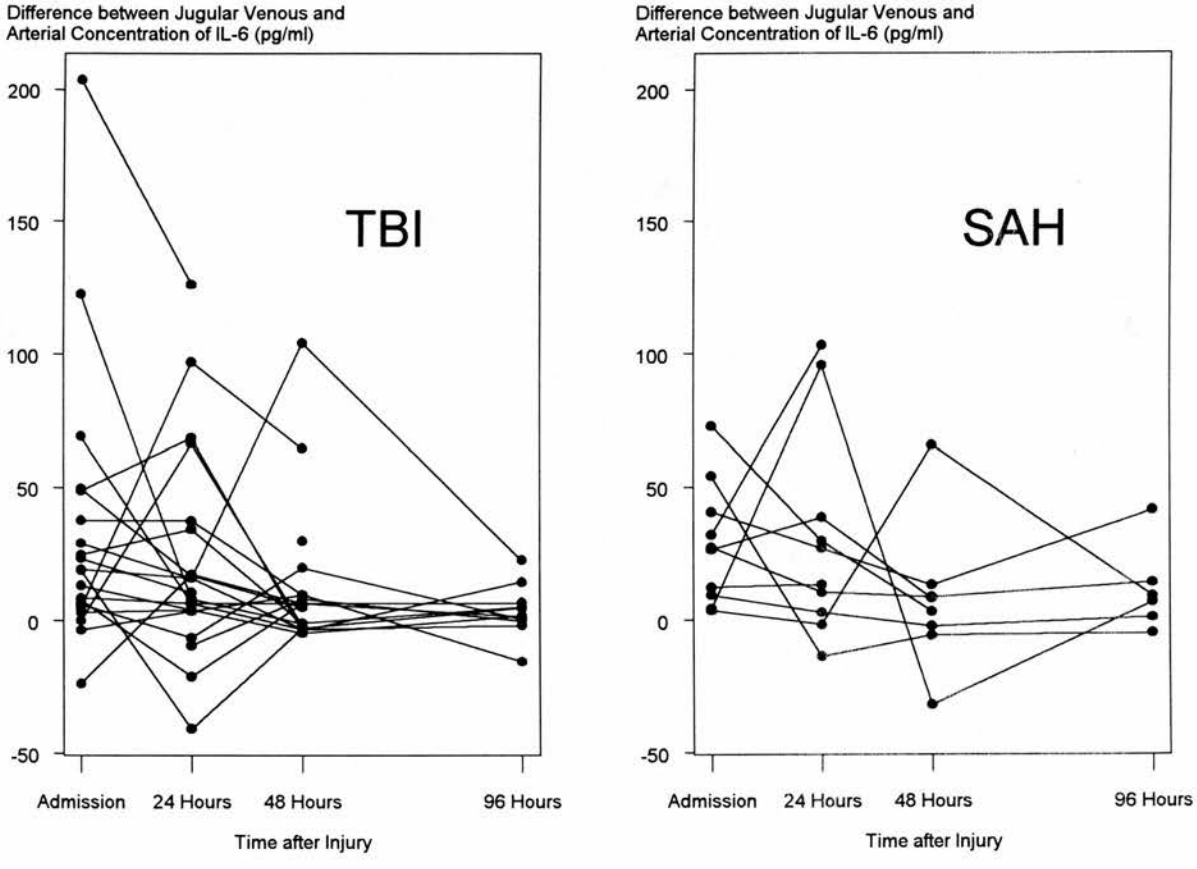
Figure 2.3 shows the individual patient profiles for transcranial IL-6 gradient for each group. In the traumatic brain injury group, median gradients at each time point were 19.2pg/ml ($p<0.001$), 10.5pg/ml ($p=0.014$), 6.3pg/ml ($p=0.018$) and 1.6pg/ml ($p=0.147$) respectively. In the subarachnoid haemorrhage group, median gradients were 26.8pg/ml ($p=0.002$), 20.1pg/ml ($p=0.027$), 5.6pg/ml ($p=0.383$) and 7.8pg/ml ($p=0.094$) respectively. There was no overall difference in IL-6 gradients across the brain between the traumatic brain injury group and the subarachnoid haemorrhage group ($p=0.350$); there were no differences between groups at any of the four sampling time points ($p=0.559$, 0.627, 0.832, 0.366 respectively).

The Adhesion Molecules

The data are presented separately for traumatic brain injury and subarachnoid haemorrhage.

sICAM-1 - Mean arterial concentration at each of the time points following injury is shown in table 2.5, together with control values from the healthy volunteers. Concentrations of sICAM-1 were not significantly different from controls in both

Figure 2.3 - Differences between jugular venous and arterial concentrations of IL-6 in traumatic brain injury and spontaneous subarachnoid haemorrhage over a 4 day period post injury.



Tables 2.5 and 2.6 - Mean concentrations of sICAM-1 and sL-selectin in arterial serum in volunteer controls and patients over a period up to 96 hours after brain injury. P values are for a two-tailed t-test of patients v controls.

	sICAM-1 (ng ml^{-1})					
Time	Traumatic Brain Injury			Subarachnoid Hemorrhage		
	Mean	95% CI	p value	Mean	95% CI	p value
Controls	254	214-295	-	254	214-295	-
Admission	267	200-335	0.778	283	208-359	0.430
24 hours	269	218-320	0.683	284	217-351	0.385
48 hours	310	252-367	0.154	331	214-449	0.178
96 hours	453	298-609	0.018	437	278-596	0.002

	L-selectin (ng ml^{-1})					
Time	Traumatic Brain Injury			Subarachnoid Hemorrhage		
	Mean	95% CI	p value*	Mean	95% CI	p value*
Controls	907	808-1006	-	907	808-1006	-
Admission	637	565-709	<0.001	600	498-702	<0.001
24 hours	590	532-647	<0.001	535	467-604	<0.001
48 hours	617	550-683	<0.001	529	424-634	<0.001
96 hours	580	488-672	<0.001	546	454-638	<0.001

groups on admission, but had risen to concentrations significantly higher than the controls by 96 hours after injury (p=0.018 for traumatic brain injury, p=0.002 for subarachnoid haemorrhage). The concentration of sICAM-1 in the traumatic brain injury group was significantly higher at 96 hours than on admission and at 24 hours (ANOVA, p=0.006, n=22). There were no significant differences between concentrations in the subarachnoid haemorrhage group with respect to time (ANOVA, p=0.080, n=10).

sL-selectin - Table 2.6 shows the mean arterial concentrations of sL-selectin for each group of patients at each time point, in addition to control data. sL-selectin concentrations were markedly below controls at all time points in both groups ($p<0.001$). There were no significant differences between concentrations of sL-selectin at any of the time points for either traumatic brain injury or subarachnoid haemorrhage.

There were no significant differences between jugular venous and arterial concentrations of either adhesion molecule in either patient group. Mean jugular venous-arterial difference for sICAM-1 in all samples was 6.9ng ml^{-1} (95% CI [-4.5-18.3], p=0.310), whilst mean jugular venous-arterial difference for sL-selectin was 3.6ng ml^{-1} (95% CI [-8.0-15.2], p=0.60). Figures 2.4 and 2.5 show the individual patient profiles for *arterial* sICAM-1 and sL-selectin in both groups of patients. There were no significant differences between the traumatic brain injury and subarachnoid haemorrhage groups at any time point for arterial or jugular venous concentrations of sICAM-1 and sL-selectin.

Figure 2.4 - Profiles of arterial sICAM-1 in the serum of patients with traumatic brain injury and spontaneous subarachnoid haemorrhage over a 4 day period post injury.

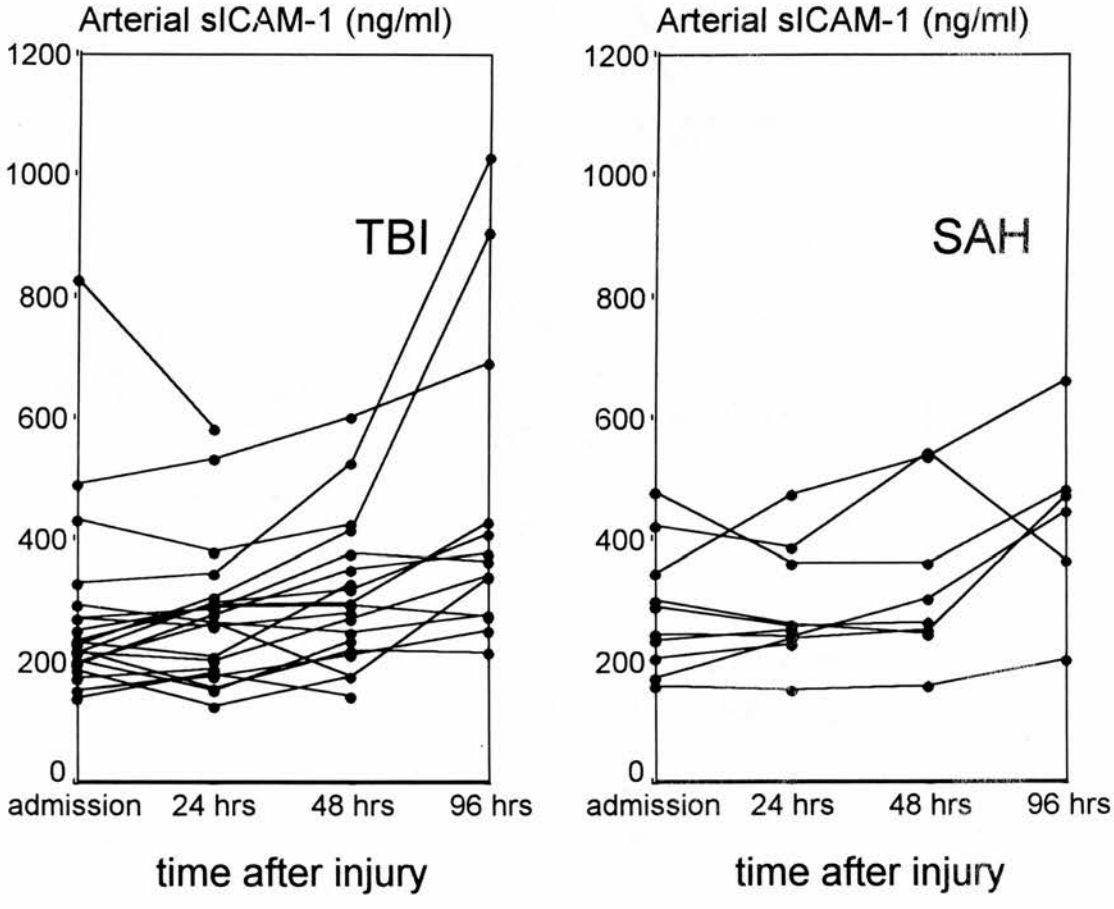
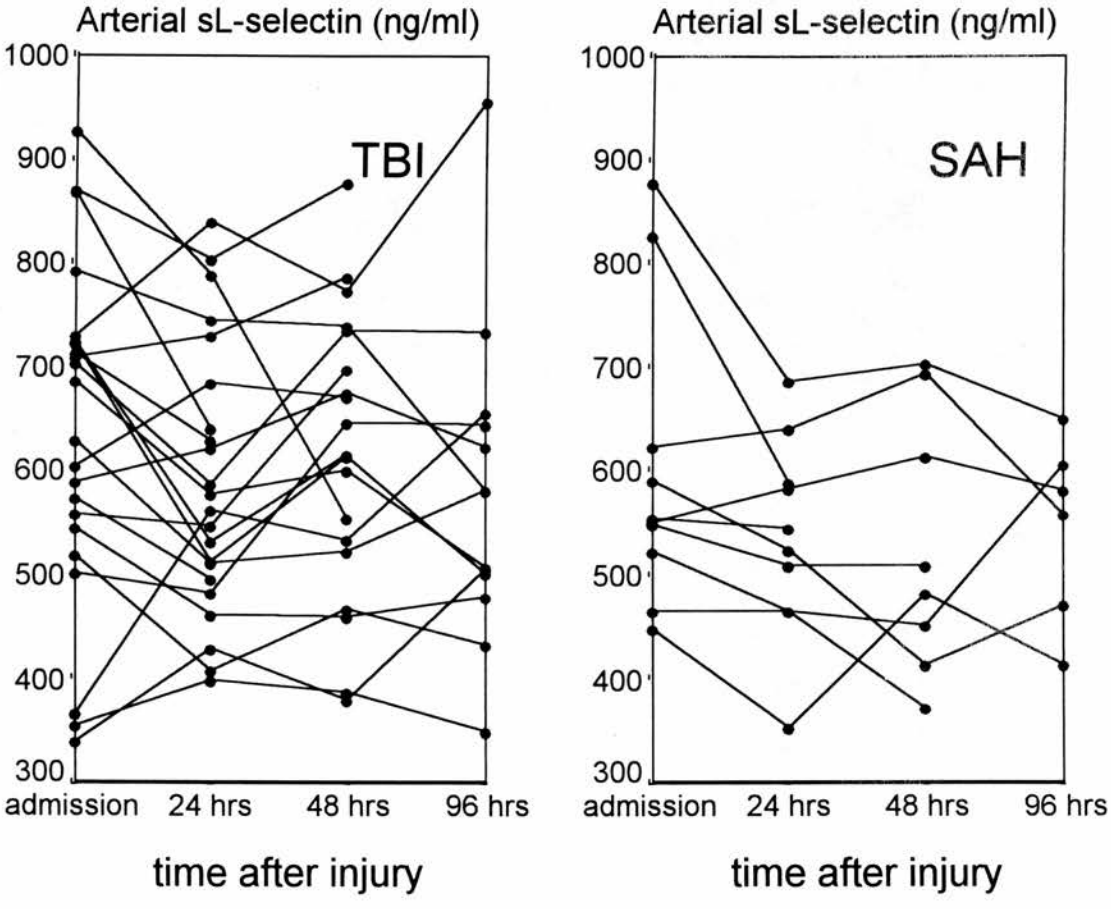


Figure 2.5 - Profiles of arterial sL-selectin in the serum of patients with traumatic brain injury and spontaneous subarachnoid haemorrhage over a 4 day period post injury.



Neuron Specific Enolase & Protein S-100

Measurements of these proteins were made in patients with traumatic brain injury only (n=21 - 1 patient in this group had insufficient serum stored for analysis of NSE and S-100).

S-100 - Mean jugular venous concentration of S-100 was 1.13 mcg/l for all patients at all time points (95% CI [0.59-1.66]), which was significantly higher than the mean arterial concentration which was 1.05 mcg/l (95% CI [0.54-1.55], p=0.022) (Figure 2.6). A breakdown of arterial concentrations at different time points, and comparisons with control samples, are shown in table 2.7. Analysis of variance showed there to be no significant differences between arterial concentrations of S-100 at different time points (p=0.90).

NSE - Mean jugular venous concentration of NSE was 11.69 mcg/l for all patients at all time points (95% CI [9.81-13.59]). This was not significantly different from the mean arterial concentration, which was 11.16 mcg/l (95% CI [9.66-12.67], p=0.416) (Figure 2.7). Table 2.7 also shows a breakdown of NSE concentrations by time. Again, analysis of variance showed there to be no significant differences between concentrations of S-100 at different time points (p=0.131).

Relationships amongst the cytokines, adhesion molecules and S-100/NSE

Firstly we looked at the relationships between pairs of measurements of the cytokines and the adhesion molecules at all time points in all patients . There was a significant positive correlation between transcranial IL-6 gradient and arterial sL-selectin concentrations (p=0.002, Figure 2.8). There was no demonstrable relationship between IL-6 gradient and arterial sICAM-1 (p=0.155), between sL-selectin and

Figure 2.6 - Boxplots comparing median, interquartile range, outliers (o) and extremes (*) for concentration of protein S-100 in jugular venous and arterial sera in patients with traumatic brain injury (p=0.022).

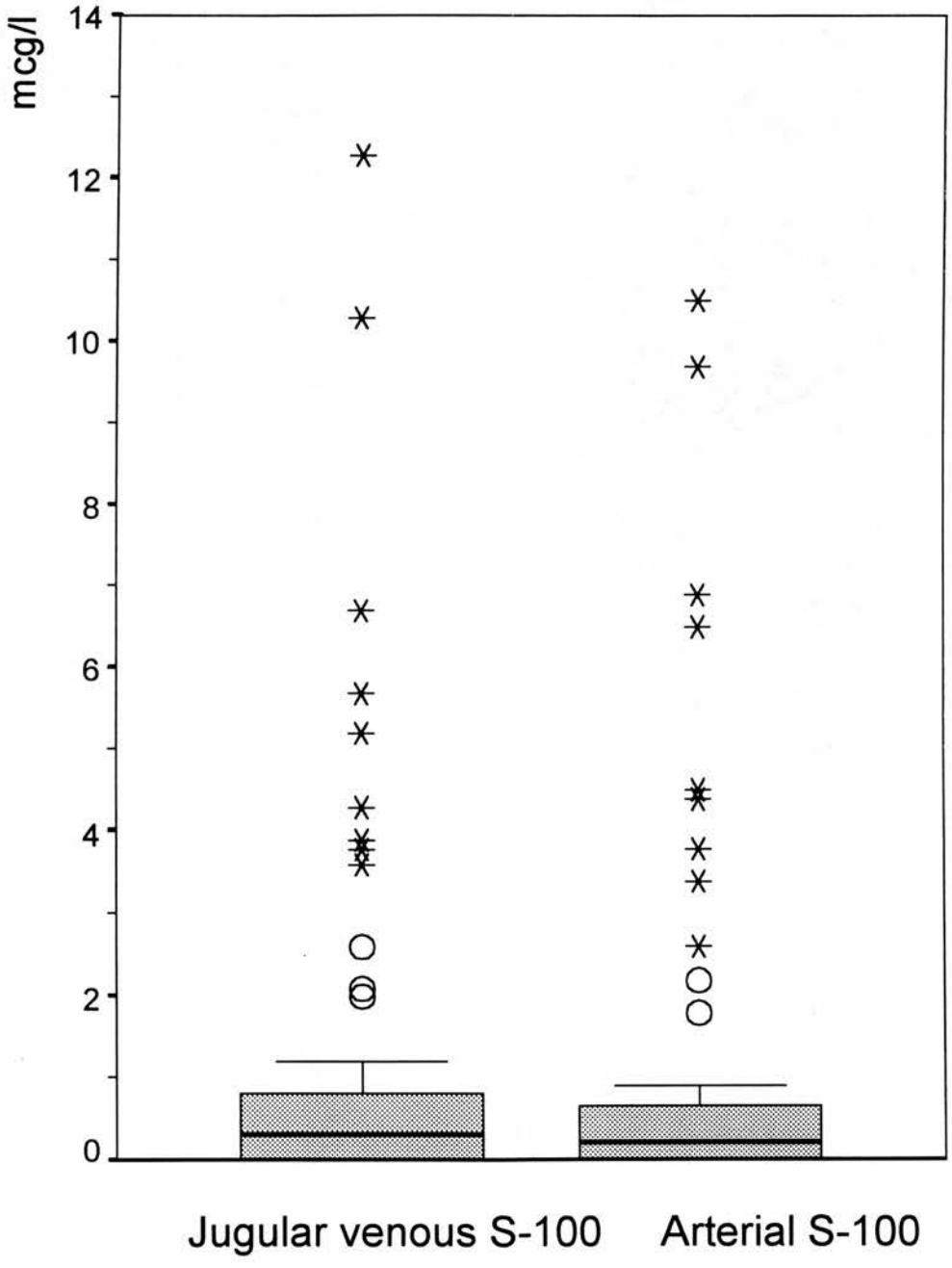


Table 2.7 - Mean concentrations of NSE and S-100 in arterial serum in volunteer controls and patients over a period up to 96 hours after brain injury. P values are for a two-tailed t-test of patients v controls.

Time	NSE (mcg/l)			S-100 (mcg/l)		
	Mean	95% CI	p value	Mean	95% CI	p value
Controls	6.9	6.3-7.5		ND	-	
Admission	13.7	10.4-17.0	<0.001	0.80	0.24-1.35	-
24 hours	11.2	8.6-13.7	0.003	1.28	0-2.49	-
48 hours	9.5	6.5-12.5	0.096	1.02	0-2.23	-
96 hours	9.2	5.4-13.0	0.220	1.15	0-2.59	-

*ND = not detected (lower limit of detection 0.2mcg/l)

Patient samples which contained concentrations of S-100 <0.2mcg/l were assigned a concentration of zero for statistical analysis.

Figure 2.7 - Boxplots comparing median, interquartile range, outliers (o) and extremes (*) for concentration of NSE in arterial and jugular venous sera in patients with traumatic brain injury (p=0.416).

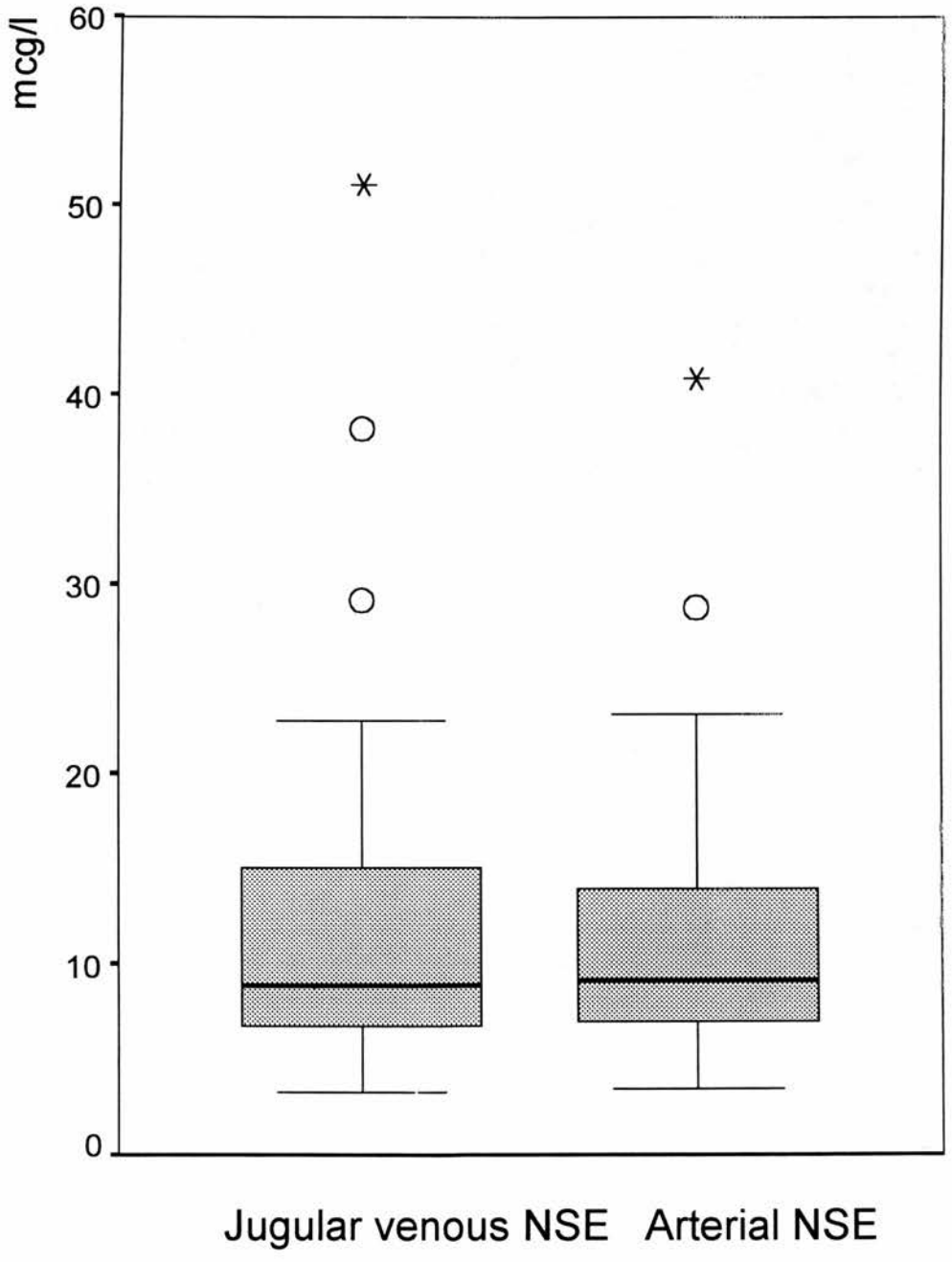
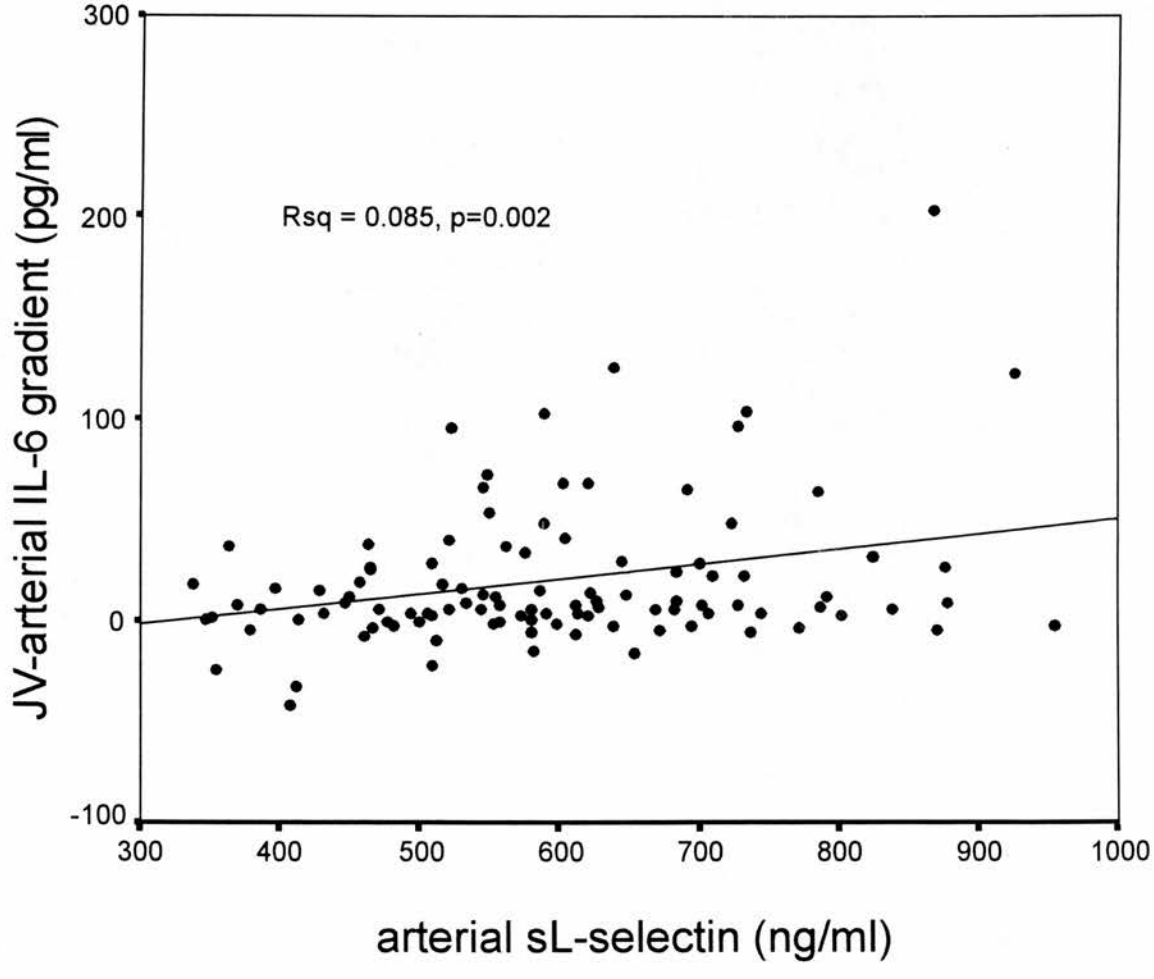


Figure 2.8 - Jugular venous-arterial IL-6 gradient plotted against arterial sL-selectin for all time points in all patients.



sICAM-1 (p=0.079) or between arterial IL-6 and either sL-selectin or sICAM-1 (p=0.909 and 0.117 respectively).

We then looked at the relationship between the proteins NSE and S-100 and the cytokines and adhesion molecules in the traumatic brain injury group. There were significant positive correlations between arterial sICAM-1 and S-100 (p=0.019, Figure 2.9), and arterial NSE and S-100 (P<0.001, Figure 2.10). There were significant negative correlations between arterial L-selectin and both NSE and S-100 (p=0.011 and 0.019 respectively, Figures 2.11 and 2.12). The r^2 values for these relationships were generally fairly small however and so their biological significance may not be important.

Relationships with outcome measures

Patient characteristics: Glasgow Outcome Scores were summarized into two categories - the first representing a good outcome (GOS 4-5), the second representing a poor outcome (GOS 1-3). In the traumatic brain injury group there were 12 patients with a good outcome and 9 patients with a poor outcome (1 lost to follow up). In the subarachnoid haemorrhage group, 3 patients had a good outcome and 7 had a poor outcome. There was a statistically significant difference in age between those with good and poor outcome (mean/standard error of mean was 31.3/3.8 yrs for 'good' and 45.9/3.6 yrs for 'poor', p=0.009), however there was no difference in Glasgow Coma Score between those with good outcome and those with poor (p=0.111).

Interleukin 6 and Interleukin 6 gradient: There was no significant relationship between either arterial IL-6 or transcranial IL-6 gradient and outcome (p=0.225 and 0.555 respectively), controlling for time (general linear model with interaction factor), in all patients.

Figure 2.9 - Arterial sICAM-1 plotted against arterial protein S-100 for all time points in patients with traumatic brain injury.

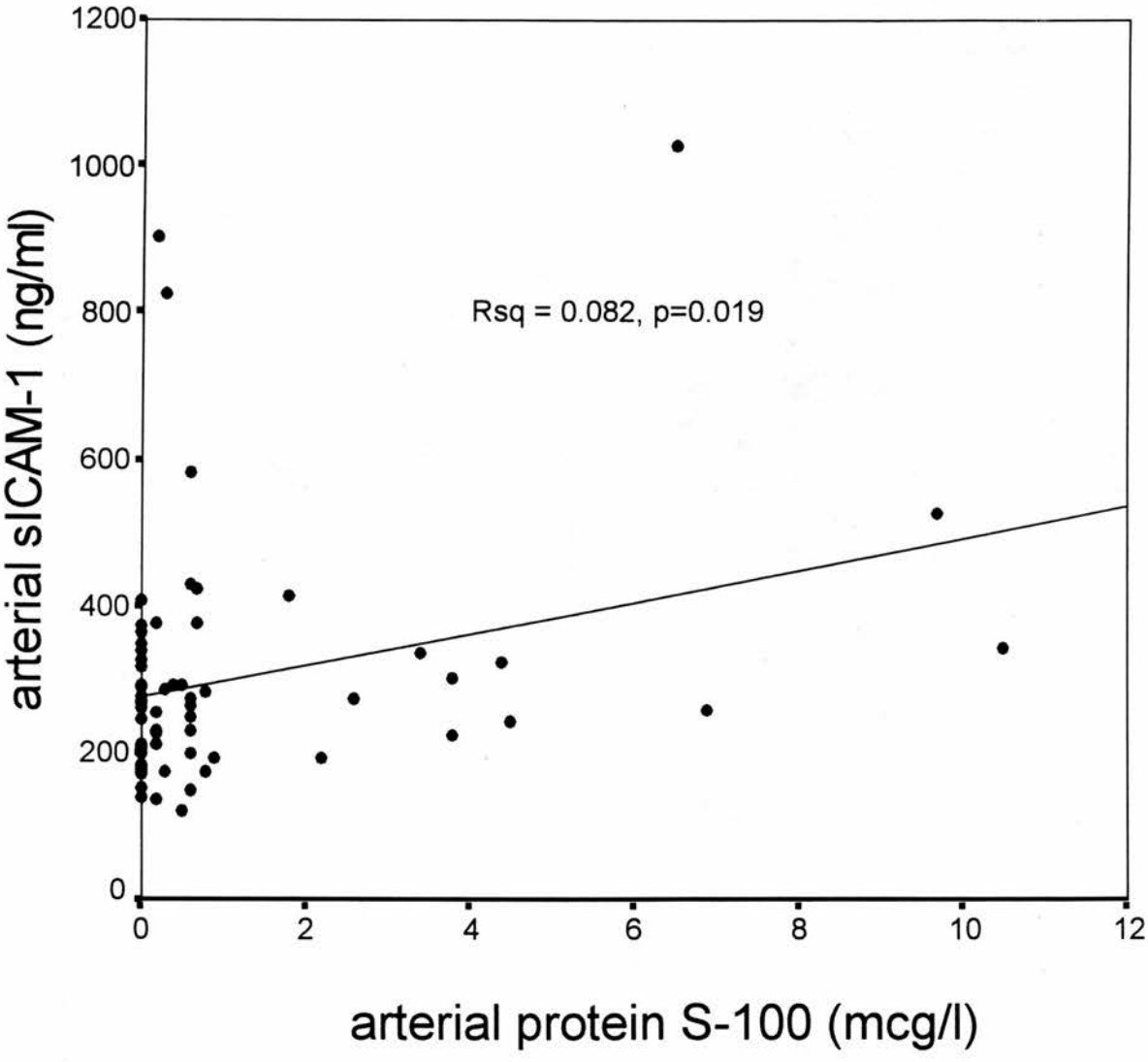


Figure 2.10 - Arterial NSE plotted against arterial protein S-100 for all time points in patients with traumatic brain injury.

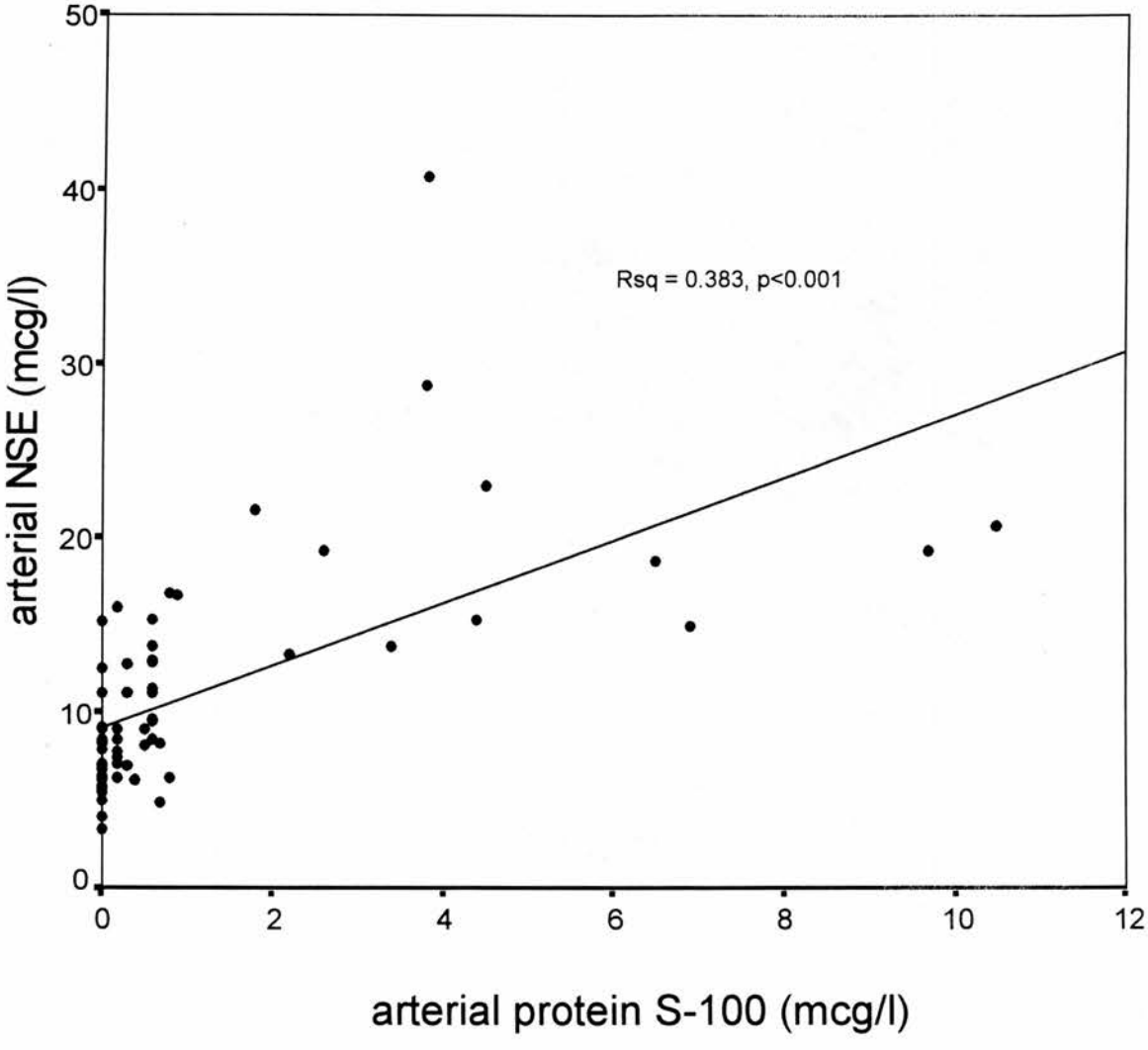


Figure 2.11 - Arterial NSE plotted against arterial sL-selectin for all time points in patients with traumatic brain injury.

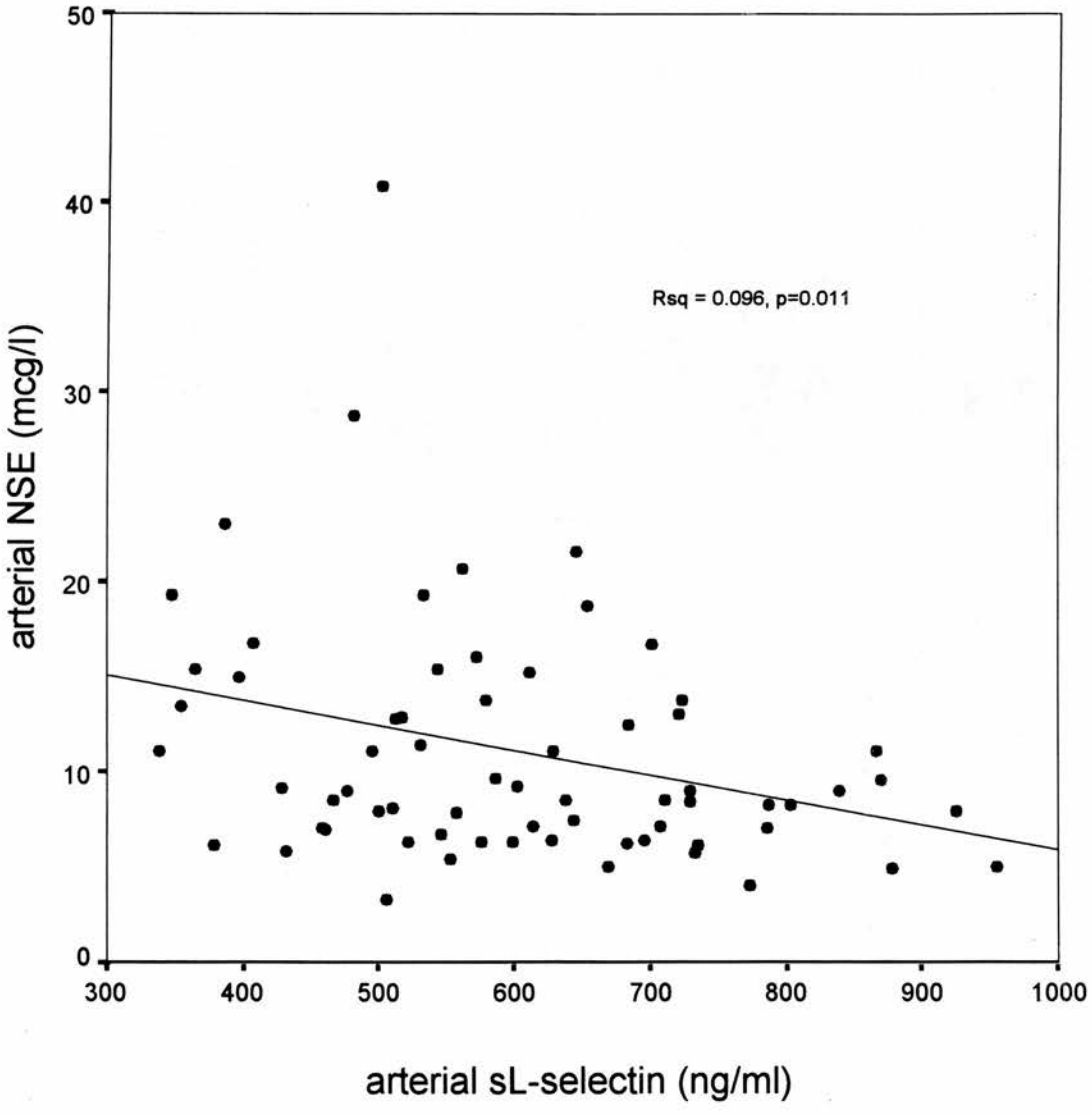
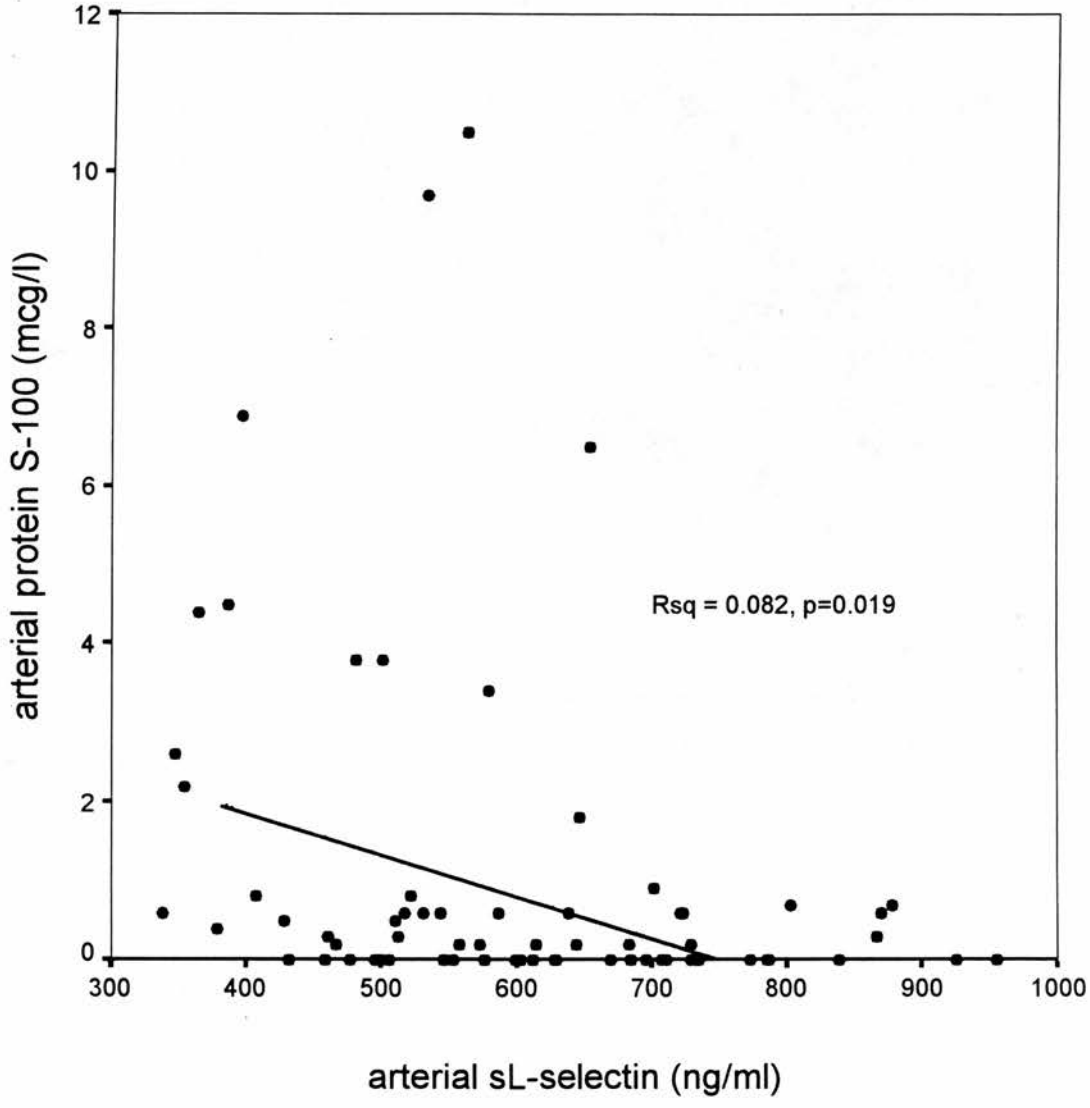


Figure 2.12 - Arterial protein S-100 plotted against arterial sL-selectin for all time points in patients with traumatic brain injury.



Adhesion molecules: There was no significant relationship between arterial sL-selectin concentrations and outcome ($p=0.053$), controlling for time, in all patients. There was a highly significant relationship between arterial sICAM-1 and outcome when all available time points were considered ($p<0.001$). Dividing the patients into traumatic brain injury and subarachnoid haemorrhage groups, sICAM-1 concentrations were significantly related to outcome in the traumatic brain injury group, but not in the subarachnoid haemorrhage group, although in the latter group this may be a Type 2 error ($p=0.001$ and $p=0.272$ respectively) (Figures 2.13 and 2.14). When only the concentrations on admission and at 24hrs were considered in the traumatic brain injury group, there remained a significant relationship with outcome ($p=0.014$). When we considered the relationship at only 1 time point (admission) the p value was 0.089.

NSE/S-100: These proteins were measured in the traumatic brain injury group only. There was a significant relationship between concentrations of both NSE and S-100, controlling for time, and outcome ($p=0.004$ and $p<0.001$ respectively) (Figures 2.15 and 2.16). Significance was still present if the first three time points only were used for NSE ($p=0.014$) and if the first two time points only were used for S-100 ($p=0.006$).

Relationships between injury type and severity, concentrations of IL-6, sICAM-1, sL-selectin, NSE and S-100, and outcome

As there are several measurements for each patient, a linear model has been used for statistical analysis here. Figure 2.17 shows the relationship between Glasgow Coma Score and arterial sICAM-1. There were significantly higher concentrations of arterial sICAM-1 in those with lower Glasgow Coma Scores ($p<0.001$). After dividing the patients into traumatic brain injury and subarachnoid haemorrhage groups, we found that a highly significant correlation was still seen for the former ($p<0.001$), but

Figure 2.13 - Profiles of arterial sICAM-1 in the serum of patients with traumatic brain injury. Those patients with poor outcome are shown with broken lines, those with good outcome with solid lines.

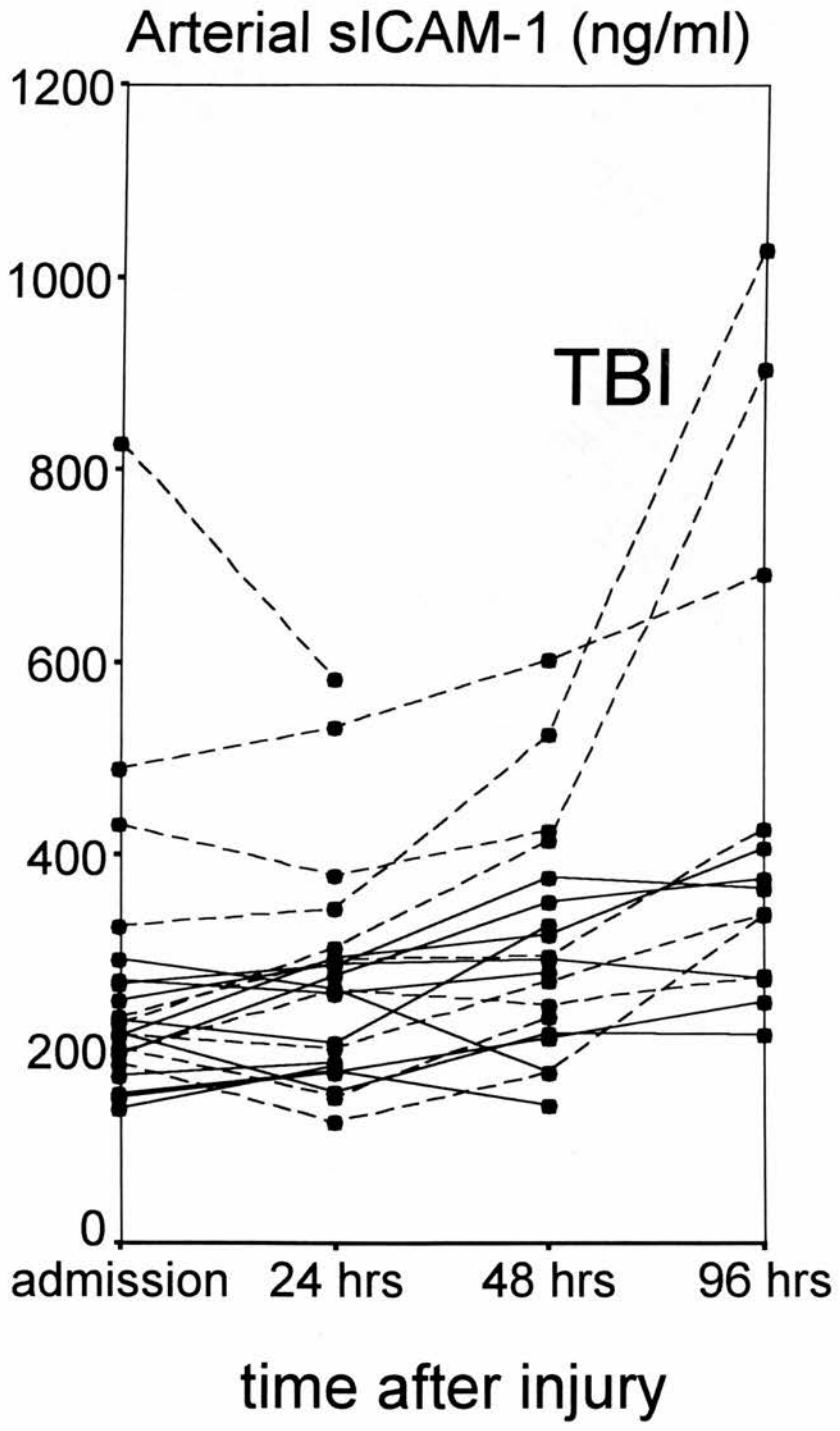


Figure 2.14 - Profiles of arterial sICAM-1 in the serum of patients with subarachnoid haemorrhage. Those patients with poor outcome are shown with broken lines, those with good outcome with solid lines.

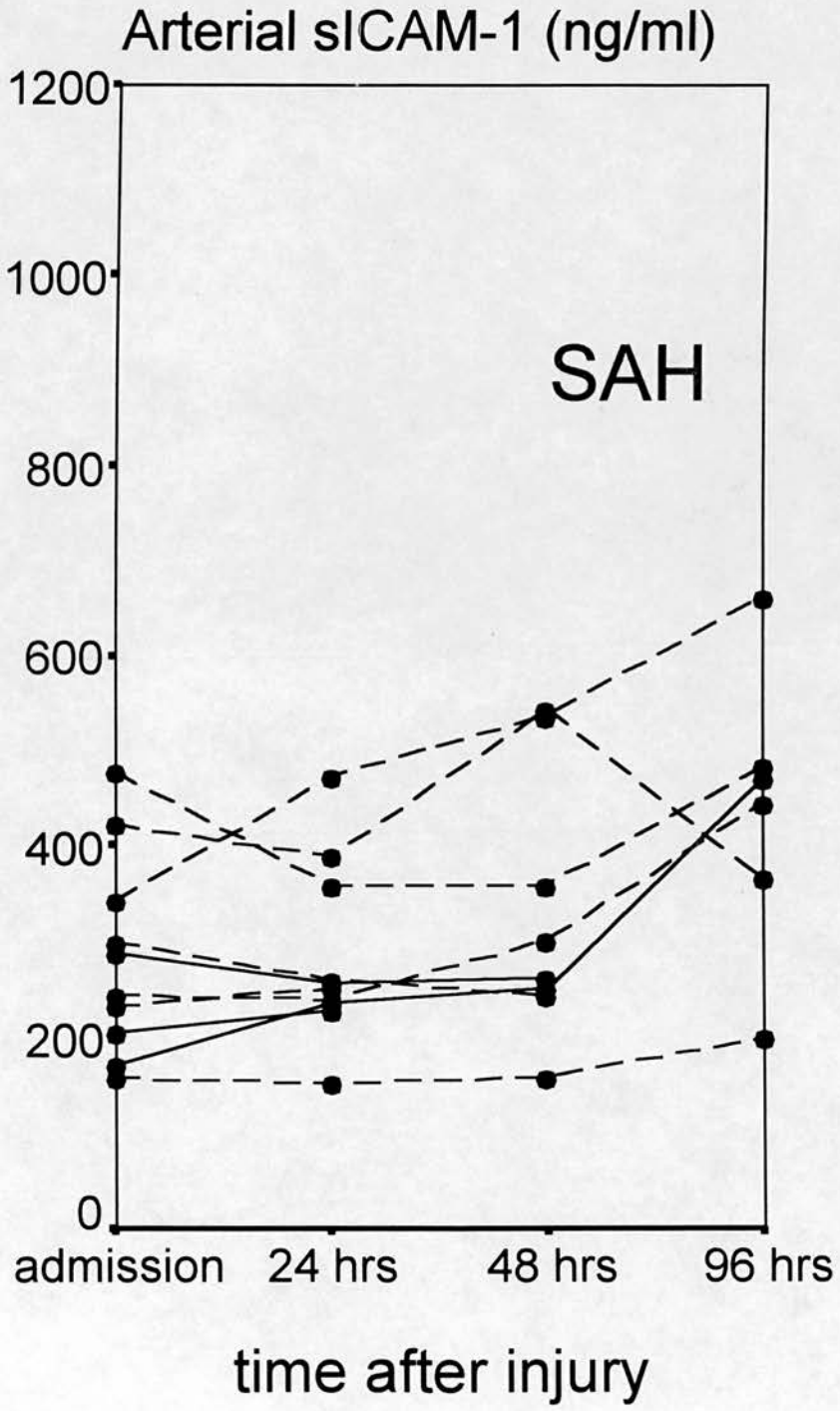


Figure 2.15 - Profiles of arterial NSE in the serum of patients with traumatic brain injury. Those patients with poor outcome are shown with broken lines, those with good outcome with solid lines.

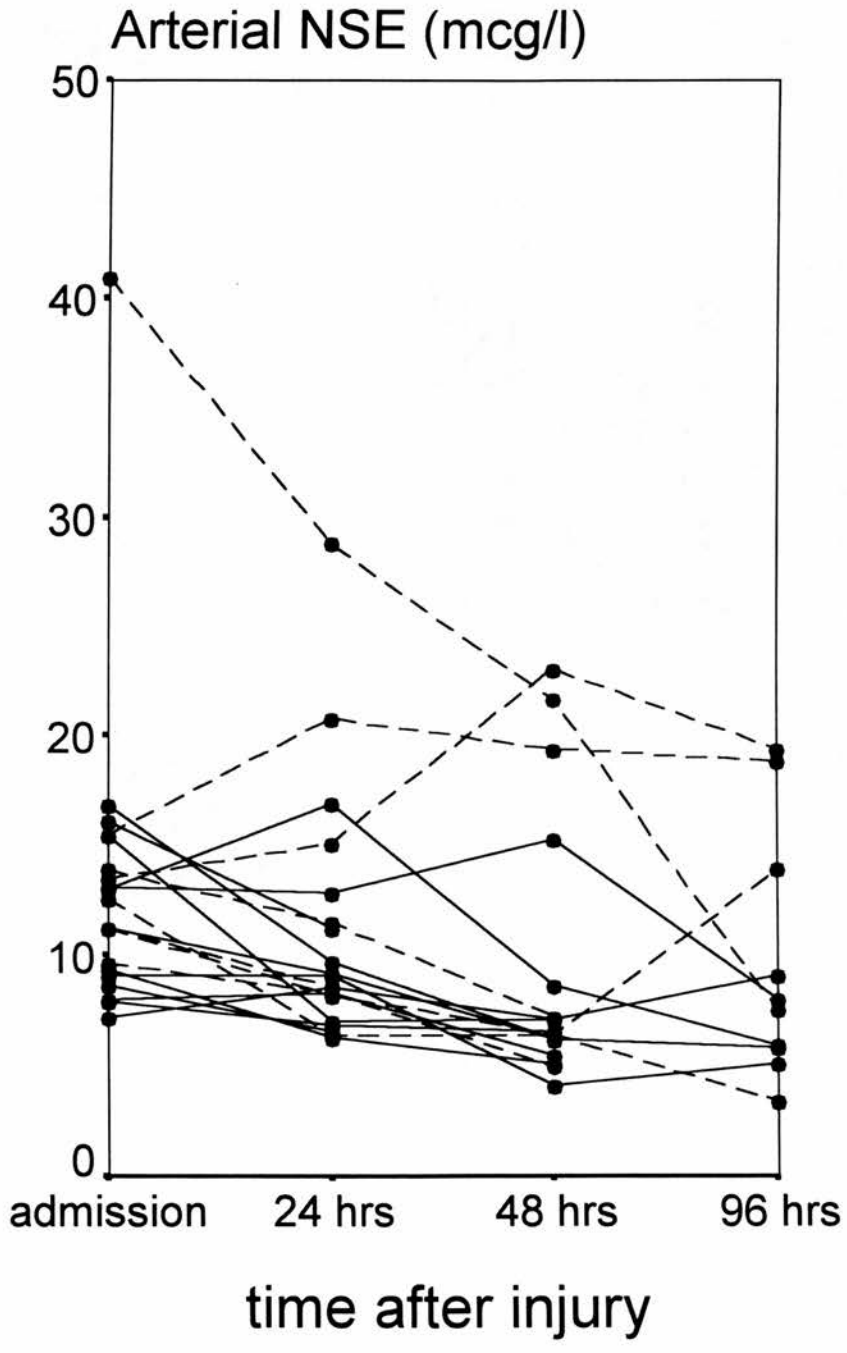


Figure 2.16 - Profiles of arterial protein S-100 in the serum of patients with traumatic brain injury. Those patients with poor outcome are shown with broken lines, those with good outcome with solid lines. In those where s-100 was undetectable (<0.2mcg/l) the profile lies on the x-axis.

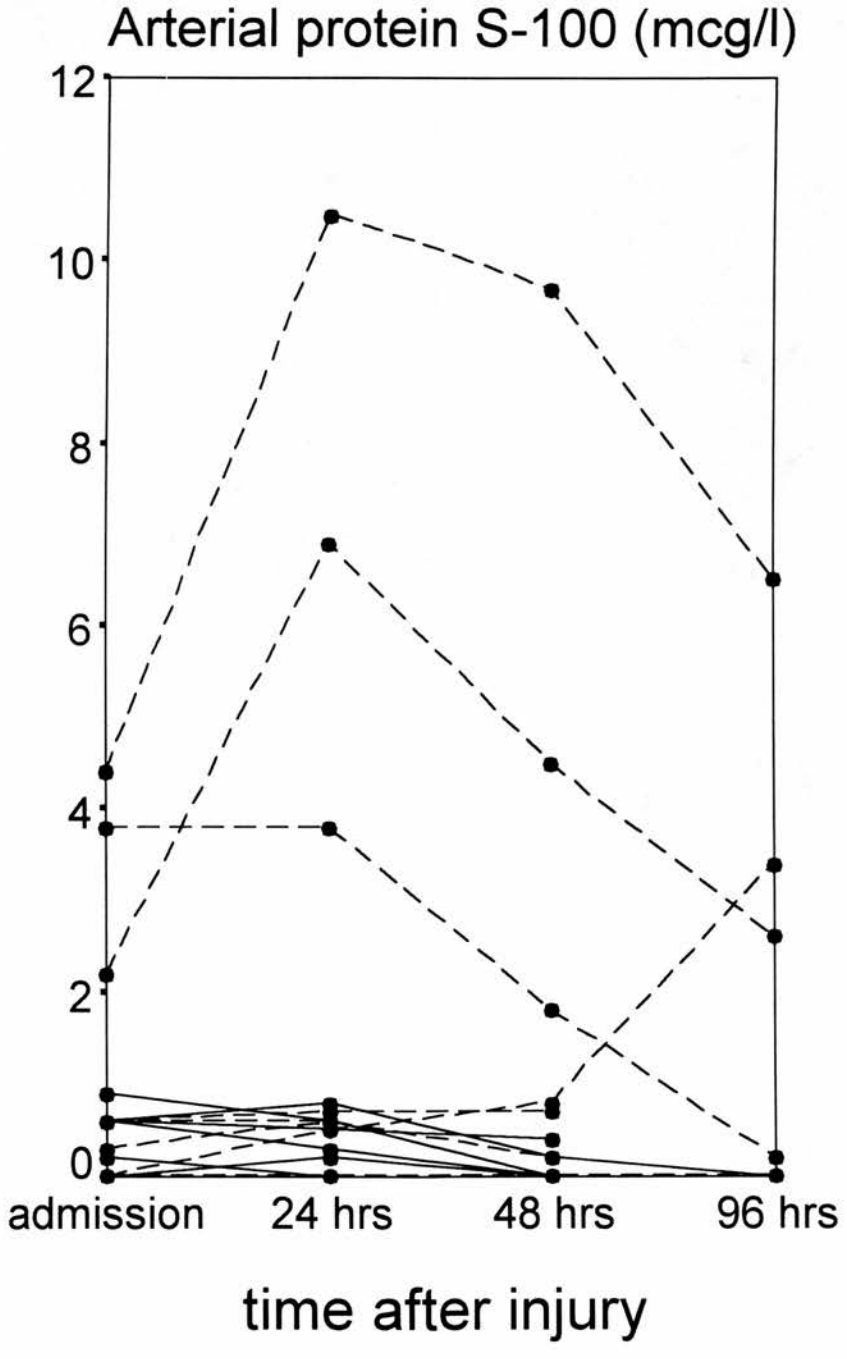
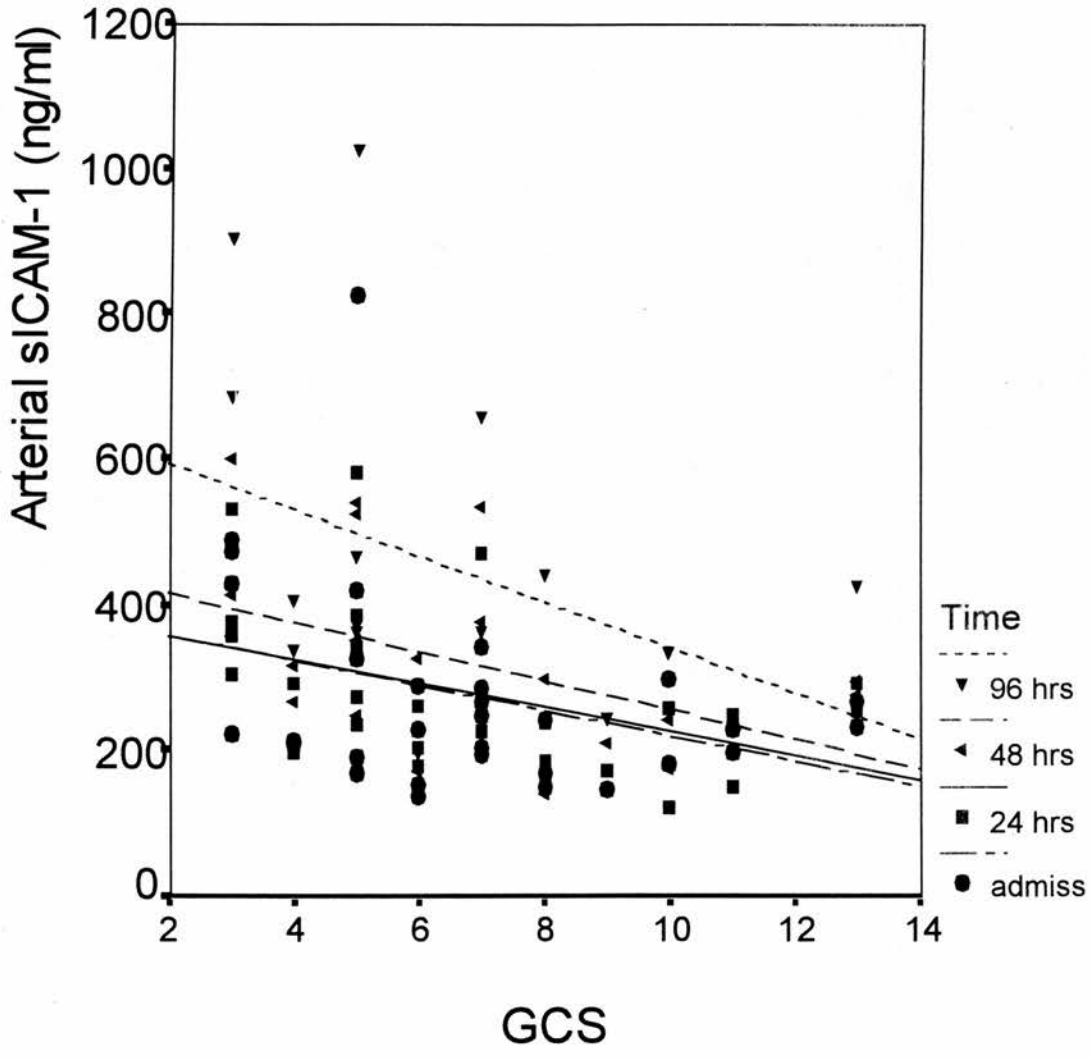


Figure 2.17 - Plot showing the relationship between arterial concentration of sICAM-1 and Glasgow Coma Score (GCS) for all patients at each time point ($p<0.001$, linear model).



the relationship was not significant in the latter (p=0.231, Figure 2.18). Surprisingly, there was also a negative correlation between arterial sL-selectin and Glasgow Coma Score (p=0.029). Again when patients were divided into traumatic brain injury and subarachnoid haemorrhage groups the correlation was significant for the former (p=0.005), but not the latter group (p=0.280). Again we must consider the possibility that because of the small number of patients in the latter group that a Type 2 error has occurred There was no significant correlation between either transcranial IL-6 gradient, arterial NSE or arterial S-100 and Glasgow Coma Score (p=0.191, 0.063 and 0.131 respectively).

We looked more closely at the type and severity of injury in the traumatic brain injury group, and in particular for the presence of extracranial injuries. 11 patients were classified as having isolated head injuries and 11 also had extracranial injuries (an Abbreviated Injury Score of >1 for a body region other than head/neck). There were no significant differences between these two groups with respect to arterial IL-6, sICAM-1, sL-selectin, NSE or transcranial IL-6 gradient (p=0.100, 0.221, 0.856, 0.813 and 0.303 respectively). There was, however a significant difference between the groups for arterial S-100, where the isolated head injury group had significantly higher arterial concentrations of S-100 than the group with additional extracranial injuries (mean/SEM = 1.91/0.58 and 0.49/0.13 mcg/l respectively, p=0.024). There was no difference in outcome between the groups (p=0.670). The mean Injury Severity Score was 22.8, SEM 1.43, range 9 - 38. There was no significant relationship between Injury Severity Score and either arterial IL-6 (Figure 2.19, p=0.063) or sICAM-1 (Figure 2.20, p=0.640), and when, for sICAM-1, only the first two time points were considered, those patients with a higher Injury Severity Score showed lower concentrations of ICAM-1, although this did not achieve statistical significance

Figure 2.18 - Plot showing the relationship between arterial concentration of sICAM-1 at each time point and Glasgow Coma Score (GCS), for patients with traumatic brain injury (top) and subarachnoid haemorrhage (bottom).

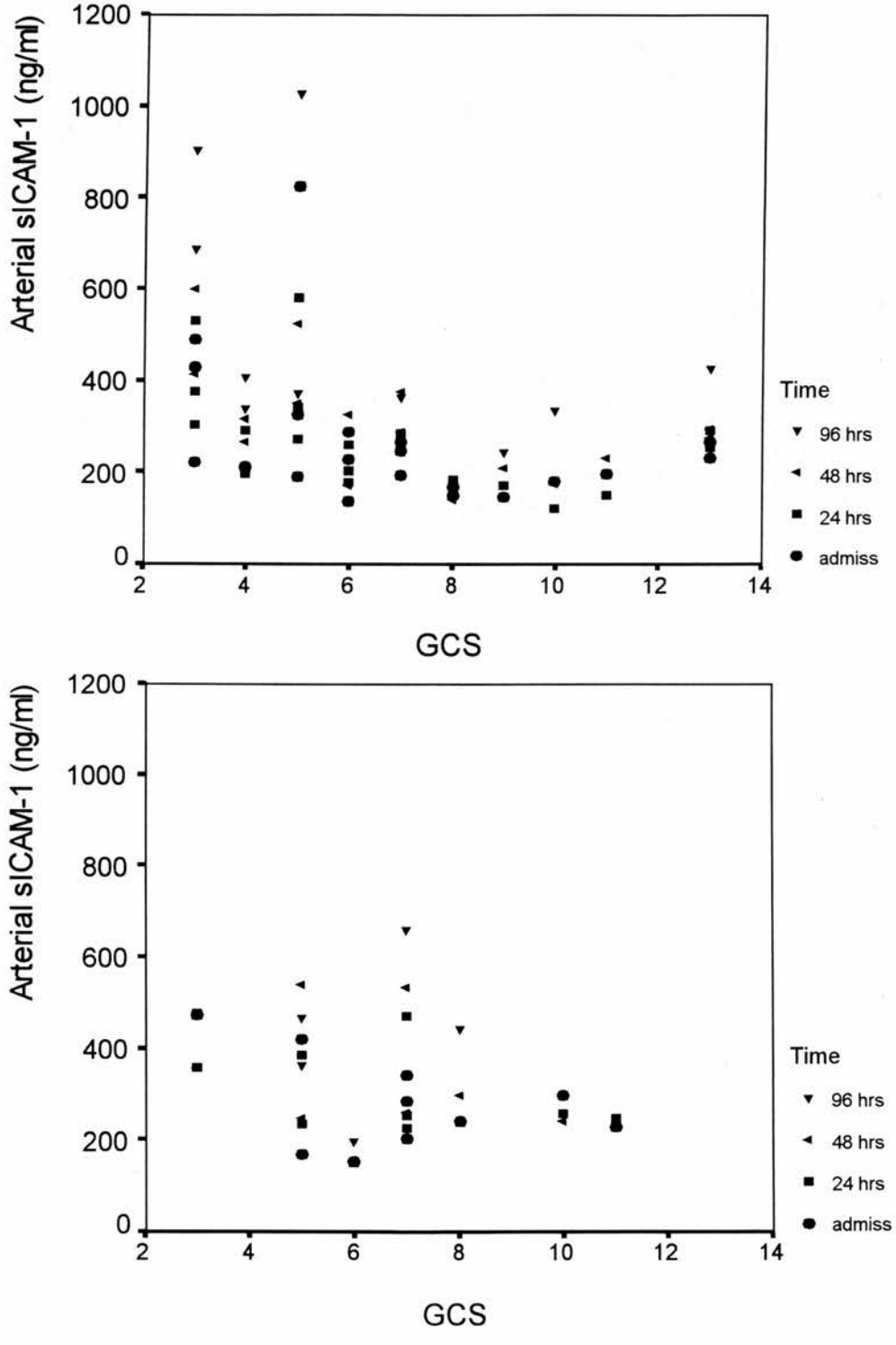


Figure 2.19 - Plot showing the relationship between arterial concentration of IL-6 and injury severity score (ISS) for all time points (p=0.063, linear model).

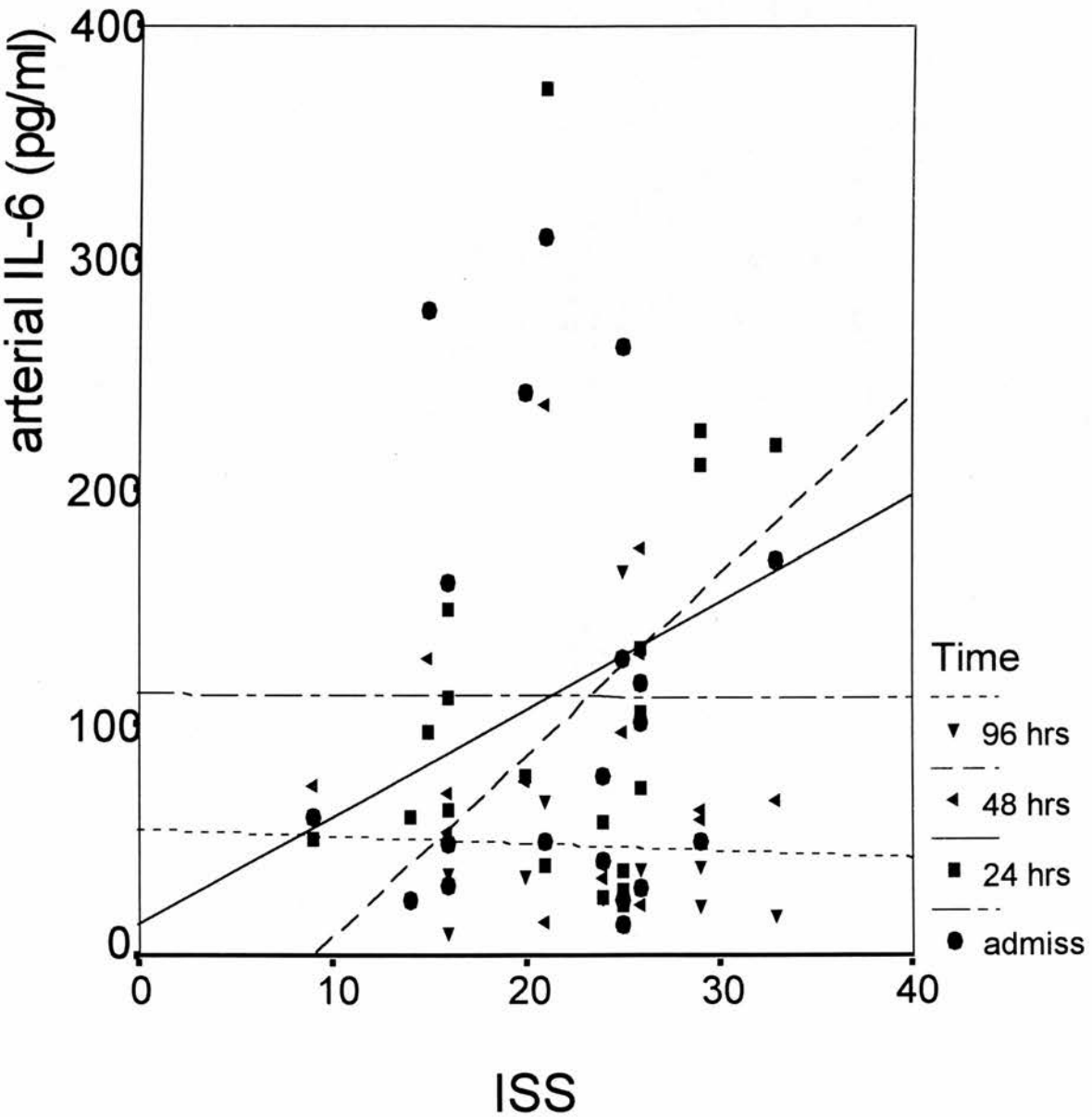
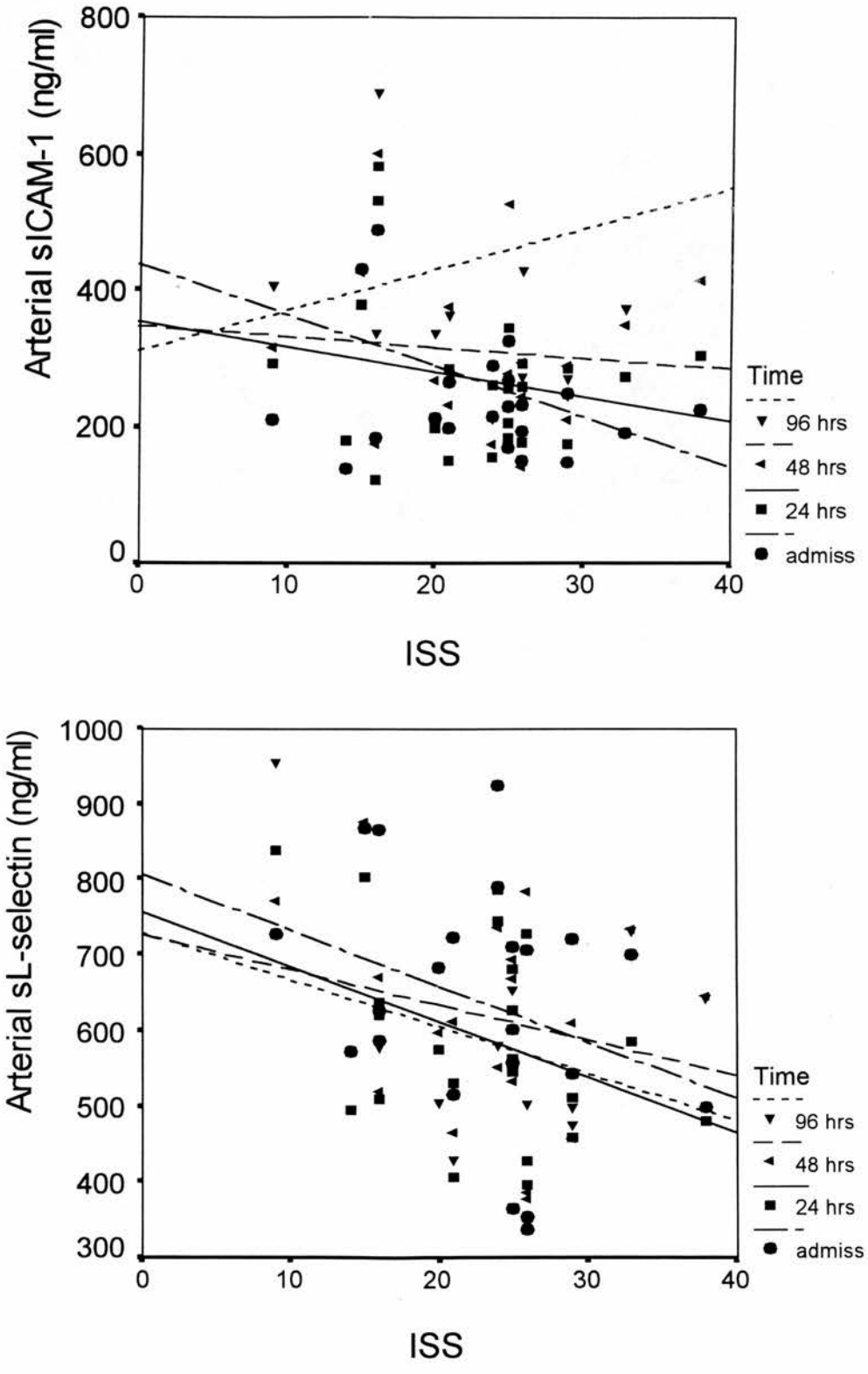


Figure 2.20 - Plots showing the relationship between arterial concentrations of sICAM-1 and Injury Severity Score (ISS) for all time points (top, p=0.64, linear model)) and for the first two time points (bottom, p=0.074).



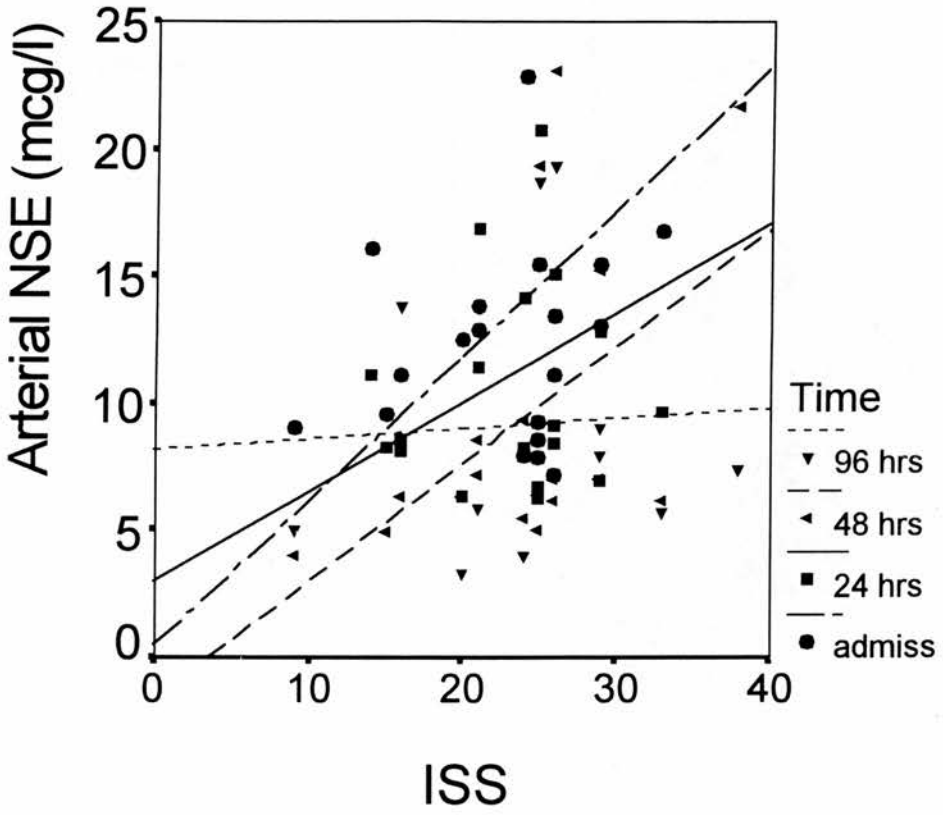
(p=0.074). There was a positive correlation between Injury Severity Score and both arterial NSE and S-100 (Figure 2.21). This was statistically significant for NSE (p=0.001), but not for S-100 (p=0.155). There was no difference in Injury Severity Score between good and poor outcome groups (mean/SEM: 23.4/1.9 and 22.0/2.5 respectively, p=0.652).

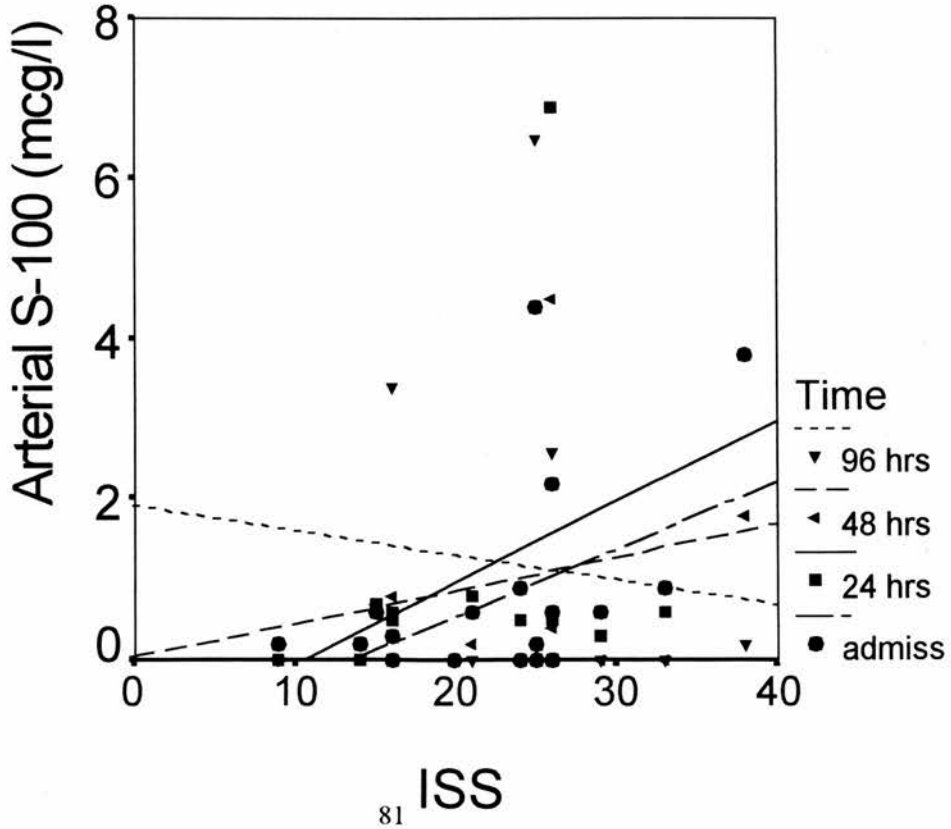
Finally we looked at type of traumatic brain injury as classified by CT scan. Scans were categorised into normal, diffuse injury, focal injury, or both. Numbers were as follows: 3 normal, 8 diffuse, 10 focal and 1 both. Statistical analysis was carried out excluding "normal" and "both" categories. There was no significant difference between focal and diffuse groups in outcome (p=0.657) and in either arterial IL-6, sICAM-1 or sL-selectin concentrations (p=0.767,0.322, 0.801 respectively). Both arterial NSE and S-100 concentrations were significantly higher in the diffuse group than in the focal group (mean/SEM: 13.1/1.5 v 9.5/0.7 mcg/l for NSE, p=0.033, and 1.20/0.31 v 0.26/0.06 mcg/l for S-100, p=0.006). In contrast, transcranial IL-6 gradients were significantly higher in the focal group than in the diffuse group (mean/SEM: 31.7/8.5 v 5.9/3.4 pg/ml, p=0.008).

Discussion

We were unable to derive any useful information from the measurement of either arterial or jugular venous serum concentrations of IL-1β, IL-8 or TNFα at these time points following acute brain injury. It is generally believed that IL-1 and TNFα are released in the very early post-injury phase[10 34], resulting in increased synthesis of IL-6, in addition to other mediators. We therefore thought it unlikely that we would find either IL-1β or TNFα in our samples, but we felt that the study would be incomplete without measuring them. It may be that we missed an early peak of

Figure 2.21 - Plots showing the relationship between arterial concentrations of NSE (top, $p<0.001$, linear model) and S-100 (bottom, $p=0.155$) and injury severity score (ISS) for all time points.





production of IL-1β and TNFα before the patients reached the intensive care unit. The relatively high lower limit of detection for IL-8 (94pg/ml) in this particular assay may have masked any significant transcranial gradient. It should also be remembered that IL-8 is found in erythrocytes and that this may confound attempts to measure its concentration accurately in serum.

Results for IL-6 show increased jugular venous concentrations of this cytokine relative to arterial concentrations, particularly within 48hrs of brain injury. Because of financial limitations it was not possible to further dilute down and re-assay the 4 pairs of samples in which IL-6 concentrations were greater than 1500pg/ml (these had already been diluted down and measured a second time). It was therefore necessary to exclude them from statistical analysis as the IL-6 concentration was not quantified.

The transcranial gradient for IL-6 on admission is around seven times higher than the highest systemic concentration measured in the controls. This suggests significant intracranial production of IL-6. The source of this increased production is likely to be glial cells, and there is experimental evidence to suggest that both astrocytes[53] and microglial cells[9] may be involved. In addition, systemic concentrations of IL-6 were greatly increased. This may be due solely to intracranial production, but is much more likely to be due to release of IL-6 from extra-cranial sources ie a mild systemic inflammatory response syndrome. Surprisingly, variation in the systemic concentrations of IL-6 was unrelated to either the Injury Severity Score or to the presence of extracranial injuries.

We have been unable to demonstrate any association between either systemic concentrations of IL-6 or transcranial IL-6 gradient with either good or poor neurological outcome, thus it may be that intracranial IL-6 production itself does not result in further neuronal damage. Indeed IL-6 may exert a protective effect as it is

known to act to reduce production of cytokines such as TNFα and IL-1β[40 147]. It does however stimulate upregulation of the activity of adhesion molecules[77 80], and this may be relevant when considering IL-6 and outcome.

We have shown that mean systemic serum concentrations of sICAM-1 are within normal limits shortly after an acute brain injury, but rise in the days following injury, becoming significantly increased between 48 and 96 hours after injury. This is in marked contrast to the profile of sL-selectin seen in systemic serum following brain injury. Surprisingly, concentrations of this molecule are significantly lower than normal values by the time of the first sample (median time 8h 30min following injury), with concentrations persistently low up to 96 hours after injury. As with transcranial IL-6 gradient, this pattern is similar in both traumatic brain injury and subarachnoid haemorrhage suggesting that, despite the different aetiology of the primary injury, the pathophysiology of the underlying inflammatory process may be similar in these conditions.

It is generally accepted that cytokines such as IL-1[148], tumour necrosis factor α[87 148 149], IL-6[80 150 151] and IL-8, are factors which regulate adhesion molecule activity. The significant differences shown between jugular venous and arterial concentrations of IL-6, were not evident however for either of the adhesion molecules studied, with jugular venous and arterial concentrations being remarkably similar. This suggests that changes in intracranial adhesion molecule activity may be the result of a systemic inflammatory response, in addition to that produced intracranially. Of course we cannot state that there is a simple relationship between the concentrations of soluble adhesion molecules present in the serum, and the activity of the membrane bound forms.

We can speculate as to the pathophysiological mechanisms behind the observed changes in adhesion molecule concentrations. It may be that ongoing damage to the endothelium, secondary to the inflammatory process, results in release of membrane bound receptors such as ICAM-1 into the blood, causing a rise in measured sICAM-1. Alternatively, upregulation of ICAM-1 production, for instance as a result of increased local concentrations of cytokines, may result in increased shedding of these receptors into the blood. These may then act to limit further adhesion by binding to leucocyte integrins and function as a type of negative feedback mechanism. The reduction in sL-selectin that we have shown may be caused by its binding to the glycoprotein endothelial counter-receptors, such as sialyl Lewis X (sLeX), on endothelium which has been "activated". Again this may be part of a negative feedback mechanism, preventing leucocyte bound L-selectin from binding to sLeX. This hypothesis is supported by the work of Spertini et al[152], who showed experimentally that sL-selectin inhibits binding of neutrophils to endothelium in a dose dependent manner.

Although we were unable to show any relationship between IL-6 (either arterial or transcranial gradient) and sICAM-1, the significant positive correlation between IL-6 gradient and arterial concentrations of sL-selectin is of interest. Looking at the initial results we expected to see a negative correlation ie high intracranial IL-6 production causing reduced serum sL-selectin. However, in those patients in whom IL-6 gradients were highest, concentrations of sL-selectin were least reduced from normal. This may again indicate that IL-6 production is in fact an attempt by the brain to antagonise the inflammatory cascade.

We showed that there is a highly significant relationship between arterial concentrations of sICAM-1 and good/poor neurological outcome. Although we

cannot show that this is a cause and effect relationship, it is a new and important finding, as rather than merely being a marker of injury, ICAM-1 is known to be a mediator of inflammation. By antagonizing the actions of ICAM-1, it may be possible to limit inflammatory secondary damage and improve patient outcome, as I have discussed at length in Chapter 1. Although mean sICAM-1 concentrations were normal on admission, Figure 2.13 shows that all patients with an admission or 24 hr concentration greater than 380 ng/ml had a poor outcome. The differences between the 48 hour and 96 hour samples are also of interest. There are only 18 patients with measurements at these time points and the result is not statistically significant, but looking at the profiles there is always a rise in sICAM-1 between these two time points in those with poor outcome.

It could be argued that the presence of extracranial injuries would result in both an increase in arterial sICAM-1 and a greater chance of a poor outcome. This was not the case in our sample - there was no correlation between Injury Severity Score and outcome, and in fact those with a higher Injury Severity Score had lower arterial concentrations of sICAM-1. Increased concentrations of sICAM-1 could not be explained by the presence or absence of extracranial injuries. This suggests that the significant increase in arterial sICAM-1 is caused by the brain injury itself, and this is supported by the fact that there is a highly significant negative correlation between arterial sICAM-1 and Glasgow Coma Score. Measurement of sICAM-1 on admission and at 24 hours after a brain injury may assist in prediction of neurological outcome.

This study supports previous work which claimed that serum NSE and protein S-100 concentrations were indicators of severe brain damage. Protein S-100 is an astroglial specific protein, and consists of two sub-units, α and β.The two forms measured in this study were the $\beta\beta$ form, found in high concentrations in glial cells

and Schwann cells, and the αβ form, found mainly in glial cells. Figure 2.16 shows that any patient in whom serum arterial S-100 was found to be above 0.9 mcg/l had a poor outcome. S-100 was undetectable in only 2 patients with poor outcome. It is interesting to note that patients with an isolated head injury had higher arterial concentrations of S-100 than patients with additional extracranial injuries. This may suggest that the brain injury itself was more severe in the group with isolated head injury. Serum concentration of NSE, an enolase restricted mainly to neurons and neuroectodermal tissue, and to some tumours, was also significantly related to outcome, although not as strongly as S-100. These proteins can be regarded as markers of brain damage. Concentrations of S-100 were highly correlated with NSE ($r=0.619$, $p<0.001$), and used together in a logistic regression model they are both independently highly predictive of poor/good outcome ($p=0.002$ and 0.027 respectively). However, as markers they are purely diagnostic or predictive tools - they are not open to manipulation or antagonism in a therapeutic sense.

Of much greater interest from the intensive care physician's point of view is any relationship between the adhesion molecules and NSE/S-100, a significant relationship adding weight to the suggestion that variations in serum adhesion molecule concentrations are associated with brain damage, and opening the window to possible drug therapies. Looking at this in our sample, it is evident that arterial concentrations of sICAM-1 are indeed significantly positively correlated with arterial concentrations of S-100, although not to NSE. In addition, both S-100 and NSE are significantly negatively correlated with arterial concentrations of sL-selectin (which fall after brain injury). These exciting results suggest that ICAM-1 in particular may be a suitable target for pharmacological antagonism in brain injured patients in the future.

It is apparent that antagonism of the mediators of the inflammatory process may play a part in future treatment of patients who have sustained an acute brain injury in the intensive care unit. Currently there is no therapeutic agent available to the intensive care physician which has been shown to be of benefit in improving outcome after an acute brain injury.

One management method currently employed in some intensive care units which may result in improved oxygenation of the brain after acute brain injury is "controlled hypertension" which involves administration of a pressor agent to raise mean arterial blood pressure to supra-normal values, and hence increase the cerebral perfusion pressure.

In the following chapter I report on the results of a study which examined indices of cerebral oxygen metabolism and blood flow in patients treated with controlled hypertension in our intensive care unit.

CHAPTER 3

CONTROLLED ARTERIAL HYPERTENSION IN THE MANAGEMENT OF ACUTE BRAIN INJURY - OBSERVATIONS OF CEREBRAL BLOOD FLOW, METABOLISM AND OUTCOME

INTRODUCTION

Cerebral blood flow (CBF) is normally coupled to and regulated by the cerebral metabolic rate for oxygen ($CMRO_2$) and functional glucose utilization[153]. If cerebral metabolic requests increase, as during seizures or fever, the cerebral blood flow increases, so that the ratio between the cerebral metabolic rate for oxygen and the cerebral blood flow remains constant ($CMRO_2$ / CBF = $AVDO_2$ where $AVDO_2$ is the arterio-jugular venous difference for oxygen).

In patients with a traumatic brain injury, the cerebral metabolic rate for oxygen is often reduced[154 155] (the normal value is 3.4 ml/100g/min (1.5 μmol/g/min)). Many of these patients have impaired homeostatic regulation of blood flow[156], and several studies[157-159] have shown wide variations in cerebral blood flow that are independent of the cerebral metabolic rate for oxygen. Lassen described such a dissociation between cerebral blood flow and metabolism as the "luxury perfusion syndrome"[160]. This syndrome is defined as excessive blood flow relative to the metabolic requirements of the brain (also known as cerebral hyperaemia).

Many authors have shown that in patients with a traumatic brain injury, reduced cerebral blood flow may cause ischaemia[157]. Miller proposed that cerebral ischaemia is the single most important cause of secondary brain injury after severe head trauma[161], a theory which was supported by the work of Jones et al.[162]. There is also histological evidence that ischaemic brain damage is common in patients with a traumatic brain injury who die[163]. Marmarou et al. analyzed The Traumatic Coma Data Bank and showed that mortality and morbidity caused by severe traumatic brain injury were strongly related to raised intracranial pressure (ICP > 20 mmHg) and hypotension (mean arterial pressure = MAP < 80 mmHg)[164]. As a result, some groups

now manage patients using a "cerebral perfusion pressure (CPP = MAP - ICP) management" approach rather than treating intracranial pressure in isolation, with emphasis on increasing arterial pressure.

Rosner et al. suggested that intracranial pressure wave behaviour is a function of unstable cerebral perfusion pressure when autoregulation of the cerebral vascular bed is relatively intact[165]. They suggested that if cerebral perfusion pressure was kept above a threshold value, periods of increased intracranial pressure classified as "plateau waves" could be avoided, and described a model of a "complex vasodilatory /vasoconstriction cascade" that is the basis for their cerebral perfusion pressure management protocol[166]. In this treatment regimen cerebral perfusion pressure is iatrogenically increased by inducing systemic hypertension.

The traditional view has been that treatment of traumatic brain injury with controlled systemic hypertension is potentially harmful because vasoparalysis, or abnormalities of autoregulation, is predominant in patients with traumatic brain injury[167 168]. Many believed that controlled systemic hypertension would potentiate uncontrollable intracranial hypertension. However, in these studies vasoparalysis occurred at very low cerebral perfusion pressures (10-20 mmHg).

A management protocol for traumatic brain injury which includes induced arterial hypertension is therefore similar to "triple H therapy" (hypertension, hypervolaemia and haemodilution)[169] for management of spontaneous subarachnoid haemorrhage. The latter method is now commonly used in the treatment of subarachnoid haemorrhage. The pathophysiological features of traumatic brain injury and subarachnoid haemorrhage are similar (as seen in Chapter 2) with broadly similar outcomes: a third of patients die, a third recover but are functionally impaired and a

third recover to independence. We have therefore included patients with traumatic brain injury and patients with spontaneous subarachnoid haemorrhage in this study.

The purpose of this observational study was to assess whether the use of controlled hypertension in our intensive care unit had adverse effects (such as increasing the incidence of cerebral hyperaemia) by examining cerebral blood flow and its relationship with metabolism, together with outcome, in a group of patients with an acute brain injury treated with controlled hypertension.

METHODS

Patients characteristics and management

Thirty two adults who had sustained either a severe closed traumatic brain injury (Glasgow Coma Score (GCS) of 8 or less without eye opening) or a spontaneous subarachnoid haemorrhage admitted to the intensive care unit at the Western General Hospital were studied. The study population included a significant number of those studied in Chapter 2. The diagnostic criterion for inclusion in the latter group was presence of subarachnoid blood on CT scanning. Data which included patient sex, age and Glasgow Coma Score after non-surgical resuscitation were collected. In addition Glasgow Outcome Scores (GOS) at 6 months after injury were obtained from information supplied by the patients' general practitioner. The Glasgow Outcome Scores refer to the following: 1 - dead, 2 - vegetative, 3 - severely disabled, 4 - moderate recovery, 5 - good recovery[144].

All the patients were managed using a protocol that emphasised prevention of secondary insults to the brain[162 170]. Patients were intubated and ventilated to maintain

an arterial partial pressure of oxygen (paO_2) greater than 13 kPa and an arterial partial pressure of carbon dioxide ($paCO_2$) of approximately 4.5 kPa.

Arterial haemoglobin oxygen saturation, ECG, rectal and peripheral temperature, systemic arterial pressure, central venous pressure and intracranial pressure were continuously monitored. The latter was monitored using an intraparenchymal fibreoptic catheter (Camino) transducer[171]. If hydrocephalus was detected in patients with subarachnoid haemorrhage, intracranial pressure was monitored with a ventriculostomy catheter (to allow CSF drainage) coupled to an external transducer system. Invasive systemic haemodynamic monitoring was universal and if clinical management dictated, the central venous catheter was replaced by a pulmonary artery catheter. Monitoring also included a fibreoptic catheter (Explorer System, Baxter Health Care Corporation, Irvine, CA, USA) in the internal jugular vein on the dominant side of cerebral venous drainage[172], positioned so that the tip was above the upper border of cervical vertebra C2 (confirmed radiologically). Cerebral venous haemoglobin oxygen saturation was displayed continuously.

Sedation and analgesia were given intravenously (alfentanil, 0.02 mg/kg/hr and midazolam, 0.07 mg/kg/hr) and if necessary a neuromuscular blocking agent was also infused (atracurium, 0.5 mg/kg/hr). Cerebral perfusion pressure was calculated as the arithmetic difference of the mean arterial pressure and the mean intracranial pressure, with the patient nursed flat. The threshold value for active cerebral perfusion pressure treatment was 70 mmHg. We aimed to establish and maintain isovolaemia or moderate hypervolaemia - a central venous pressure of 10-12 mmHg or pulmonary artery wedge pressure of 14-16 mmHg was used as a guideline. In

patients with subarachnoid haemorrhage this clinical management met the criteria for "triple H therapy".

In a proportion of patients a vasopressor (noradrenaline, adrenaline or methoxamine) was infused to maintain a cerebral perfusion pressure of at least 70 mmHg[166]. Every effort was made to minimize the dosage of vasopressor to reduce possible side effects. Mannitol was given in some patients (together with frusemide and a plasma expander) with intracranial hypertension, mainly for its haemodynamic and microrheological effects, rather than to effect cerebral dehydration[173]. Barbiturates were given only if intracranial hypertension was refractory to this regimen. Cerebral blood flow measurements were not made during barbiturate infusion.

Physiological measurements

Cerebral blood flow, together with arterial and jugular venous haemoglobin oxygen saturation, lactate and glucose, was measured at least daily during the period of intracranial pressure monitoring (range of measures 0 - 4 days after injury). A total of 79 measurements of cerebral blood flow (range 1-7 per patient) were made.

Cerebral blood flow was measured by the nitrous oxide saturation method[174] with the modification described by Scheinberg[175]. 3% nitrous oxide was introduced into the patient's inspired gases via the low pressure inlet of a Servo Ventilator (900C, Siemens, Sweden) and a continuous side-stream infrared monitor (Capnograph II, Datex, Helsinki, Finland) was used to check that the nitrous oxide administration rate was constant throughout the ten minute period required for brain tissue to become saturated with nitrous oxide. During the ten minutes of gas inhalation, simultaneous samples (called "arterial integrated" and "venous integrated"

respectively) were drawn continuously from the radial artery and jugular bulb into pre-heparinized syringes at the rate of 0.86 ml/min. Accuracy was maintained during aspiration by placing the two syringes in a parallel infusion/withdrawal pump (Harvard Apparatus Ltd., Edenbridge, UK). When collection of the integrated samples was complete, administration of nitrous oxide was continued for another 10 seconds whilst 4 ml samples (called respectively "arterial final" and "venous final") were withdrawn simultaneously from the artery and the jugular vein.

After ten minutes of nitrous oxide inhalation, the internal carotid blood and brain nitrous oxide concentrations should be in diffusion equilibrium, and thus the final venous sample represented the cerebral nitrous oxide concentration[175]. The final arterial sample was an internal check of the veracity of the procedure. This measurement should be in close agreement with the final venous sample if equilibrium between arterial and cerebral tissue is present. We then injected each blood sample through a gas tight butyl rubber septum into a 20 ml glass (pre-vacuumed) vial using a 25 gauge hypodermic needle; the blood samples were kept at room temperature until the nitrous oxide concentration was measured. To ensure that nitrous oxide in the gaseous phase was in equilibrium with that in the blood sample, a vortex was created by agitating each vial for 30 seconds (Vortex Genie 2 Mixer, Merck House)[175]. The gas above the blood sample in the vial was then sampled and its nitrous oxide concentration measured by an infrared analyzer (Nitrous Oxide Analyzer, ADC Series 7000, Analytical Development Co, Ltd, Hoddesdon, UK) with an accuracy to within 1 part per million.

Cerebral blood flow was then calculated according to the following equation:

$$CBF = [\ Fv / (Ai - Vi) \times time\ ] \times 100$$

The numerator is the cerebral venous concentration in parts per million at equilibrium (Fv); the denominator is the difference between arterial and venous integrated samples in parts per million multiplied by the time of "wash in" (duration of aspiration of integrated samples). Multiplication of the result by 100 gave the cerebral blood flow in units of mls/100g/min. We obtained simultaneous arterial and jugular venous samples at the end of the ten minute period for measurement of paO_2, $paCO_2$, pH, haemoglobin, glucose and lactate concentrations and haemoglobin oxygen saturation. PaO_2, $paCO_2$ and pH were measured with a Corning blood gas analyzer (Ciba Corning Diagnostic Ltd, Halstead, Essex, UK). Haemoglobin concentration and haemoglobin oxygen saturation were measured with a Corning 270 laboratory co-oximeter (Ciba Corning Diagnostic Ltd, Halstead, Essex, UK). Blood lactate and glucose concentrations were measured with a lactate-glucose analyzer (YSI Incorporated Yellow Springs, Ohio, USA).

The cerebral metabolic rate for oxygen was calculated by multiplying the cerebral blood flow by the arterio-jugular venous difference for oxygen. The normal arterio-jugular venous difference for oxygen is 6.3 ml/dl (2.8 μmol/ml)[154]; the normal cerebral metabolic rate for oxygen determined by this method is 3.4 ml/100g/min (1.5 μmol/g/min). The lactate-oxygen index (LOI) was calculated with the following equation:

$$LOI = -AVDL / AVDO_2$$

where AVDL is the the arterio-jugular venous difference for lactate (both AVDL and $AVDO_2$ are expressed in molar units). The lactate-oxygen index is normally less than 0.03, whilst values of 0.08 or more indicate increased cerebral lactate production[154]. The cerebrovascular resistance (CVR, which indicates vascular tone) was also calculated using the formula:

$$CVR = CPP / CBF$$

Reliable cerebral blood flow measurements require a physiological steady-state for at least 10 minutes and therefore to avoid delay in cerebral perfusion pressure management, studies were performed only during periods of stability.

We also carried out transcranial Doppler ultrasound insonation (EME TC2 - 64 B Uberlingen, Germany) of the middle cerebral artery during measurement of cerebral blood flow, using the method described by Aaslid et al.[176], on the same side of the head as the intracranial pressure monitor was situated. We chose the depth of insonation that gave the highest mean flow velocity, and took measurements from an average of at least 15 cardiac cycles. The average mean flow velocity from the middle cerebral artery measured in our unit was 60 ± 7 (SD) cm/sec[177]. In patients with a brain injury, the mean flow velocity was considered abnormally high if it exceeded 100 cm/sec.

Reproducibility of measurement of nitrous oxide concentration in blood

We determined the reproducibility of the measurement technique in five subjects by taking 10 separate blood samples from the arterial and jugular venous catheters during the period of administration of nitrous oxide, after saturation of the

brain with nitrous oxide. To determine the within patient variability of the procedure, we then fitted a one-way analysis of variance model to remove patient difference, leaving a residual standard deviation of 3.65ppm. When divided by the fitted mean value of 112.8ppm, this gave a coefficient of variation of 3.2%.

Classification of measurements and patients

Measurements of cerebral blood flow were corrected, assuming a 3% change in cerebral blood flow per mmHg change in $paCO_2$[155], and normalized to a $paCO_2$ of 4.5 kPa (34 mmHg). The arterio-jugular venous difference for oxygen also varies with arterial carbon dioxide tension, and therefore was corrected by the same factor. The normal arterio-jugular venous difference for oxygen at a $paCO_2$ of 4.5 kPa (34 mmHg) is 6.3 + (6 * 0.03 * 6.3) = 7.4 ml/dl, where "6" represents the difference between normal arterial $paCO_2$ and the target arterial $paCO_2$ (40 - 34) for our patients in mmHg.

As cerebral hyperaemia is defined as "blood flow in excess of metabolic requirements", our primary classification of measurements was by the arterio-jugular venous difference for oxygen. If cerebral metabolism and blood flow are coupled, an inverse relationship between the arterio-jugular venous difference for oxygen and cerebral blood flow should exist. When cerebral blood flow is reduced relative to metabolic demand, a wide arterio-jugular venous difference for oxygen is found, until a threshold of 9 ml/dl is reached, which suggests hypoperfusion. If cerebral blood flow is in excess of metabolic demand, the arterio-jugular venous difference for oxygen is narrow. Each measurement (n=79) was classified by the value of the arterio-jugular venous difference for oxygen: hyperaemia was defined as an arterio-

jugular venous difference for oxygen of less than 4 ml/dl; non-hyperaemia was defined as an arterio-jugular venous difference for oxygen of greater than 4 ml/dl[178].

In addition, individual patients (n=32) were categorized as hyperaemic if one or more of the measured arterio-jugular venous differences for oxygen was classified as hyperaemic during the study period. If all measurements of arterio-jugular venous differences for oxygen were in the non-hyperaemic classification, the patient was categorized as non-hyperaemic.

All results are expressed as mean ± standard error of the mean. Inter-group comparison was by one-way analysis of variance (ANOVA) or Fisher's exact test for two by two tables. Statistical analysis was carried out with "SPSS Base 7.0 for Windows".

RESULTS

Data of patient characteristics are shown in Table 3.1. Study patients with subarachnoid haemorrhage were significantly older than those with a traumatic brain injury. A mass lesion was detected in 31% of patients with traumatic brain injury. The data for both subarachnoid haemorrhage and traumatic brain injury are presented together in a similar manner to that of Robertson et al.[156].

Cerebral perfusion pressure and cerebral blood flow

A primary goal in clinical management was to avoid systemic hypotension, and active treatment to increase cerebral perfusion pressure was started if cerebral perfusion pressure fell below 70 mmHg. At the time of the study most of the patients

Table 3.1 - Characteristics of the 32 patients studied. Data are expressed as mean ± SEM.

	TBI	SAH
sex (M/F)	15/4	8/5
age	37 ± 4	50 ± 4†
CTM score I - II	n = 13	
CTM score mass lesion	n = 6	
GCS on admission	6 ± 1	7 ± 1

Definition of abbreviations:
TBI = traumatic brain injury
SAH = subarachnoid hemorrhage
CTM score = computed tomography Marshall score
GCS = Glasgow Coma Scale

† t-test ($p = 0.03$)

had a cerebral perfusion pressure of between 70 and 120 mmHg. There was no demonstrable relationship between cerebral perfusion pressure and cerebral blood flow, but this was to be expected from the autoregulatory curve.

A vasopressor was used during 56% of measurements (Table 3.2). In those being treated with a vasopressor, 32.4% of measurements of arterio-jugular venous differences for oxygen were classified as hyperaemic, compared with 24.1% of those not being treated with a vasopressor. This difference was not statistically significant (p=0.586). There was no evidence of ischaemia by either arterio-jugular venous difference for oxygen or cerebral blood flow measurement criteria[155 156] (Table 3.3). This result was supported by the fact that the lactate oxygen index did not show increased cerebral lactate production in any case.

Hyperaemia v non-hyperaemia (measurements)

The comparison between hyperaemic and non-hyperaemic measurements is presented in Table 3.3. The two measurement groups were equivalent with respect to cerebral perfusion pressure, intracranial pressure, mean arterial pressure and mean flow velocity. $PaCO_2$ was higher in the hyperaemic measurement group but this was not statistically significant. Similarly, cerebral blood flow was higher in the hyperaemic measurement group, but this did not achieve statistical significance. Although the mean flow velocity was similar in hyperaemic and non-hyperaemic measurement groups, both were higher than the normal reference range (46-74 cm/sec). This supports the findings of Chan et al.[178] and confirms that it is common to find increased mean flow velocities in patients with acute brain injury.

The lactate oxygen index was significantly different between measurement groups, but in both groups it was lower than the limit previously described for

Table 3.2 - Relationship between hyperaemia and vasopressor treatment. Data was not available for 13 measurements.

	Hyperaemic measurements	Non-hyperaemic measurements	Total
Treated with vasopressor	12	25	37
No vasopressor	7	22	29
Total	19	47	66

Table 3.3 - Comparison of cerebral blood flow and metabolic parameters between hyperaemic and non-hyperaemic measurements (n=79). Data are expressed as mean ± SEM.

Parameter	Hyperaemic measures	Non-hyperaemic measures	p value
CBF (ml/100g/min)	116.7 ± 11.0	93.1 ± 7.6	0.081
$AVD0_2$ (ml/dl)	3.17 ± 0.14	5.70 ± 0.14	< 0.001†
CPP (mmHg)	79.3 ± 2.3	79.7 ± 2.0	0.895
ICP (mmHg)	17.1 ± 1.7	14.0 ± 1.2	0.158
MAP (mmHg)	96.4 ± 3.1	93.8 ± 1.7	0.424
$paC0_2$ (kPa)	4.37 ± 0.12	4.14 ± 0.01	0.085
$CMR0_2$ (ml/100gr/min)	3.74 ± 0.38	8.38 ± 1.72	0.011†
LOI (µmol/ml)	-0.030 ± 0.014	0.005 ± 0.008	0.015†
MFV (cm/s)	87.1 ±10.5	89.2 ± 7.4	0.897
CVR (mmHg/100g/min	0.80 ± 0.07	1.02 ± 0.09	0.043†

Definition of abbreviations:
CBF = cerebral blood flow
$AVD0_2$ = arterio-jugular venous oxygen difference
CPP = cerebral perfusion pressure
ICP = intracranial pressure
MAP = mean arterial pressure
$paC0_2$ = arterial partial pressure of carbon dioxide
$CMR0_2$ = cerebral metabolic rate of oxygen
LOI = lactate oxygen index
MFV = mean flow velocity
CVR = cerebral vascular resistance

CBF and $AVD0_2$ are corrected for $PaC0_2$ = 34 mmHg
† significant results (Student's t-test)

ischaemia[179]. The cerebrovascular resistance was lower in the hyperaemic measurement group, as a result of higher cerebral blood flow measurements, where cerebral perfusion pressures were similar in both groups. There was also a significant difference in the cerebral metabolic rate for oxygen between measurement groups. In normal subjects, cerebral blood flow and arterio-jugular venous difference for oxygen vary inversely, with a constant cerebral metabolic rate for oxygen, however in the hyperaemic measurement group, the mean arterio-jugular venous difference for oxygen was lower per unit cerebral blood flow. The result was that the cerebral metabolic rate for oxygen in the hyperaemic measurement group was lower than in the non-hyperaemic measurement group.

Hyperaemia v non-hyperaemia (patients)

If we examine the results when patients are categorized as hyperaemic or non-hyperaemic we find similar results (Table 3.4). The main difference is that there was a significant difference in mean arterial pressure (and hence cerebral perfusion pressure) between patient groups. Mean arterial pressure was higher in the non-hyperaemic patients than in the hyperaemic patients ($p=0.010$).

Coupling between cerebral blood flow and metabolism

If coupling between blood flow and metabolism is preserved, an inverse relationship between arterio-jugular venous difference for oxygen and cerebral blood flow is evident. Our data confirmed this relationship in the non-hyperaemic measurement group, in accordance with cerebral blood flow and arterio-jugular venous difference for oxygen parameters (Figure 3.1). There is an inverse relationship between cerebral blood flow and arterio-jugular venous difference for oxygen on the

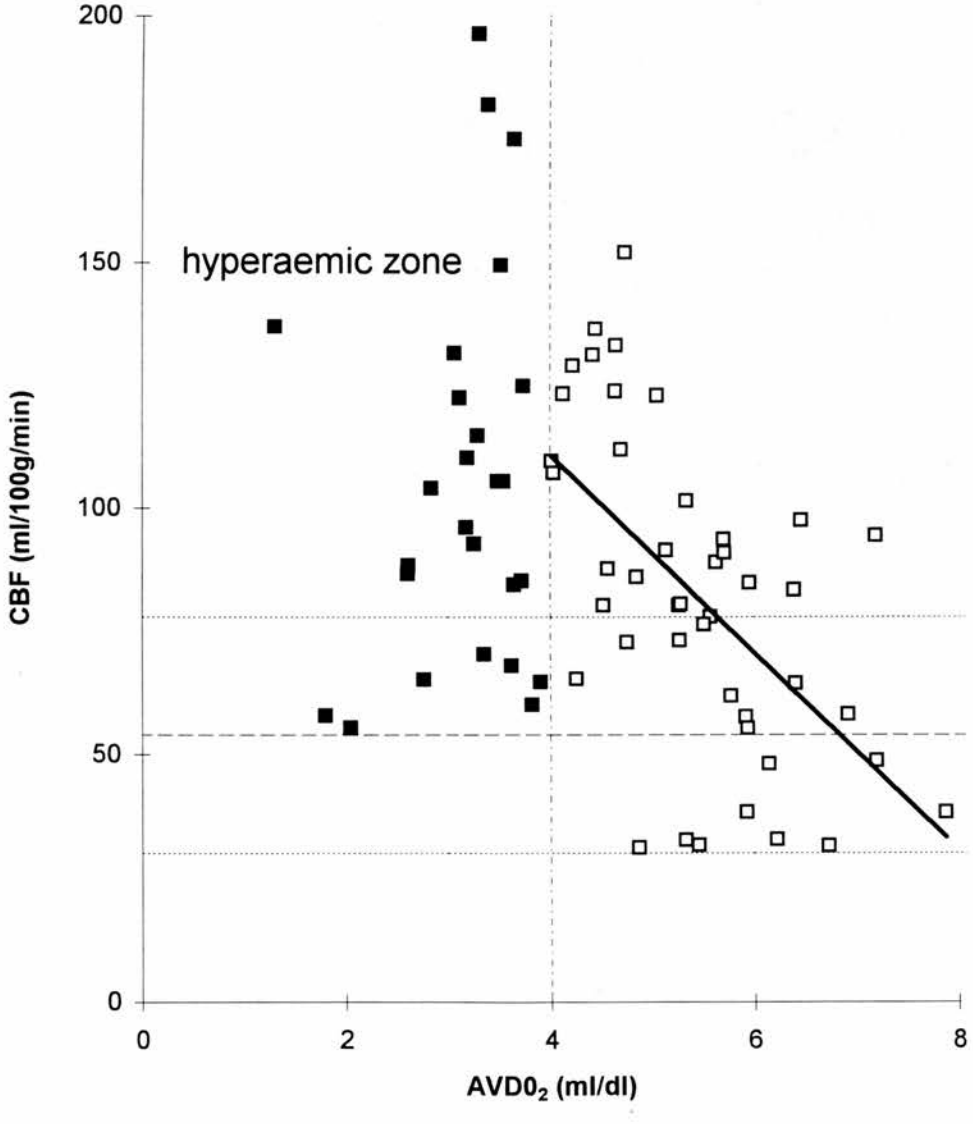
Table 3.4 - Comparison of cerebral blood flow and metabolic parameters between patients categorized as hyperaemic and non-hyperaemic (n=32). Data are expressed as mean ± SEM.

Parameter	Hyperaemic patients	Non-hyperaemic patients	p value
CBF (ml/100g/min)	109.3 ± 8.4	90.1 ± 9.5	0.145
$AVD0_2$ (ml/dl)	3.94 ± 0.20	5.95 ± 0.15	< 0.001†
CPP (mmHg)	75.4 ± 1.8	83.9 ± 2.4	0.006†
ICP (mmHg)	15.4 ± 1.3	14.6 ± 1.6	0.689
MAP (mmHg)	90.8 ± 2.2	98.5 ± 1.6	0.010†
$paC0_2$ (kPa)	4.19 ± 0.09	4.23 ± 0.08	0.719
$CMR0_2$ (ml/100gr/min)	4.16 ± 0.34	9.85 ± 2.39	0.023†
LOI (µmol/ml)	-0.020 ± 0.012	0.001 ± 0.010	0.270
MFV (cm/s)	95.7 ± 7.2	81.3 ± 10.1	0.250
CVR (mmHg/100g/min	0.82 ± 0.06	1.09 ± 0.11	0.040†

Definition of abbreviations:
CBF = cerebral blood flow
$AVD0_2$ = arterio-jugular venous oxygen difference
CPP = cerebral perfusion pressure
ICP = intracranial pressure
MAP = mean arterial pressure
$paC0_2$ = arterial partial pressure of carbon dioxide
$CMR0_2$ = cerebral metabolic rate of oxygen
LOI = lactate oxygen index
MFV = mean flow velocity
CVR = cerebral vascular resistance

CBF and $AVD0_2$ are corrected for $PaC0_2$ = 34 mmHg
† significant results (Student's t-test)

Figure 3.1 - Cerebral blood flow (CBF) plotted against arterio-jugular venous difference for oxygen ($AVD0_2$): hyperaemic measurements ($AVDO_2$< 4ml/dl) are shown as solid squares, non-hyperaemic measures ($AVDO_2$> 4ml/dl) as open squares. Dotted lines indicate the normal range for CBF. There is no relationship between the parameters in the hyperaemic measurement set (p=0.776). There is a negative correlation between parameters in the non-hyperaemic measurement set (r=-0.288, p=0.042)



right-side of the graph (p=0.042); this relationship is absent in the hyperaemic measurement group (left-side of the graph).

Cerebral blood flow and cerebral metabolic rate for oxygen

The relationship between cerebral blood flow and cerebral metabolic rate for oxygen in the two measurement groups is shown in Figure 3.2. The non-hyperaemic measurement group shows the expected relationship between the two variables and confirms preserved coupling. The hyperaemic group shows a similar relationship but this is described by a different fit line. The increased cerebral blood flow measurements are not related to a change in arterio-jugular venous difference for oxygen (Figure 3.1) suggesting that "luxury perfusion" is present[160].

Cerebral blood flow, metabolism and vascular tone

The relationship between cerebral blood flow and cerebrovascular resistance is shown in Figure 3.3. All the points are on the same line and show a relationship between the two parameters described by the following exponential equation:

$$y = a\,(Ln)x + b$$

where y is the cerebral blood flow and x is the cerebrovascular resistance. However, the hyperaemic measurement group is displaced in the steeper part of the curve suggesting passive behaviour of the vessels, dependent on the cerebral blood flow. The relationship between vascular tone (cerebrovascular resistance) and cerebral metabolism (cerebral metabolic rate for oxygen) is shown in Figure 3.4. The calculated cerebrovascular resistances of the hyperaemic measurement group are on a

Figure 3.2 - Cerebral blood flow (CBF) plotted against the cerebral metabolic rate for oxygen ($CMRO_2$). Non-hyperaemic measurements (open squares) show the expected coupling between cerebral blood flow and metabolism (dashed line, $r=0.94$, $p<0.001$). Hyperaemic measurements (closed squares) show similar behaviour but shifted upwards (continuous line, $r=0.90$, $p<0.001$).

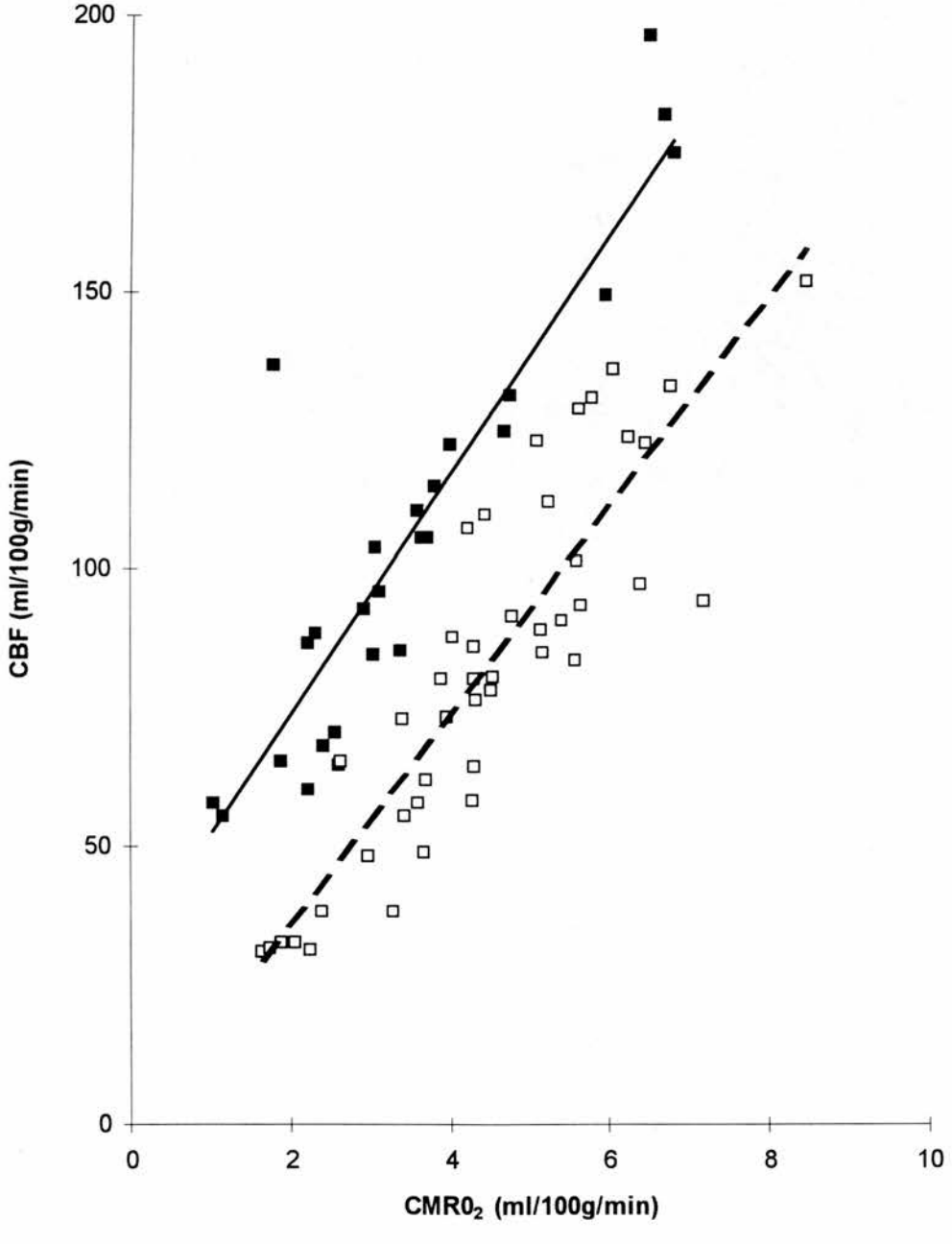


Figure 3.3 - Cerebral blood flow (CBF) plotted against cerebrovascular resistance (CVR). Data points from both measurement groups lie around the same regression line ($r^2 = 0.82$, $p<0.001$) but the hyperaemic group (closed diamonds) is displaced from the non-hyperaemic group (open squares) in the steeper part of the curve.

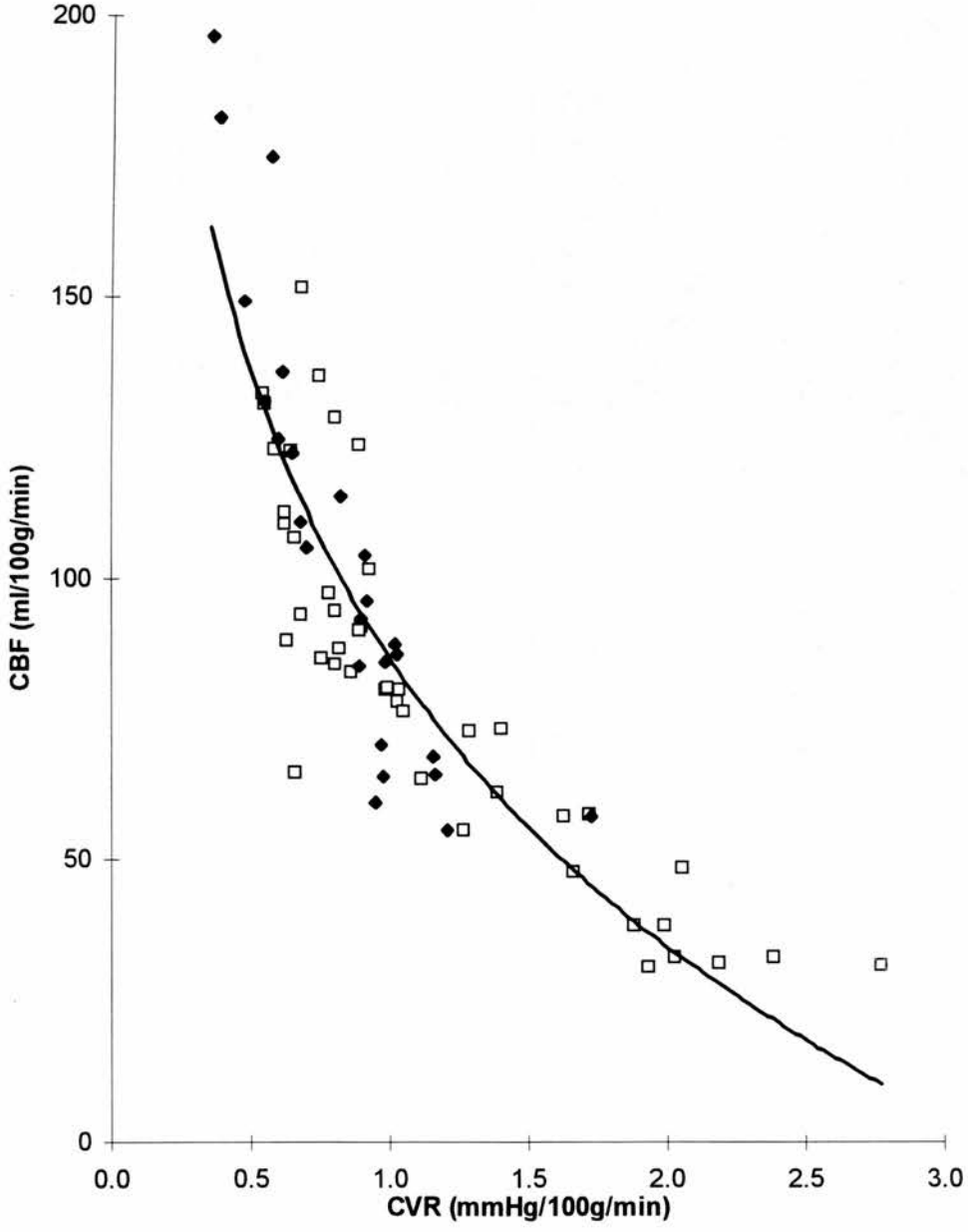
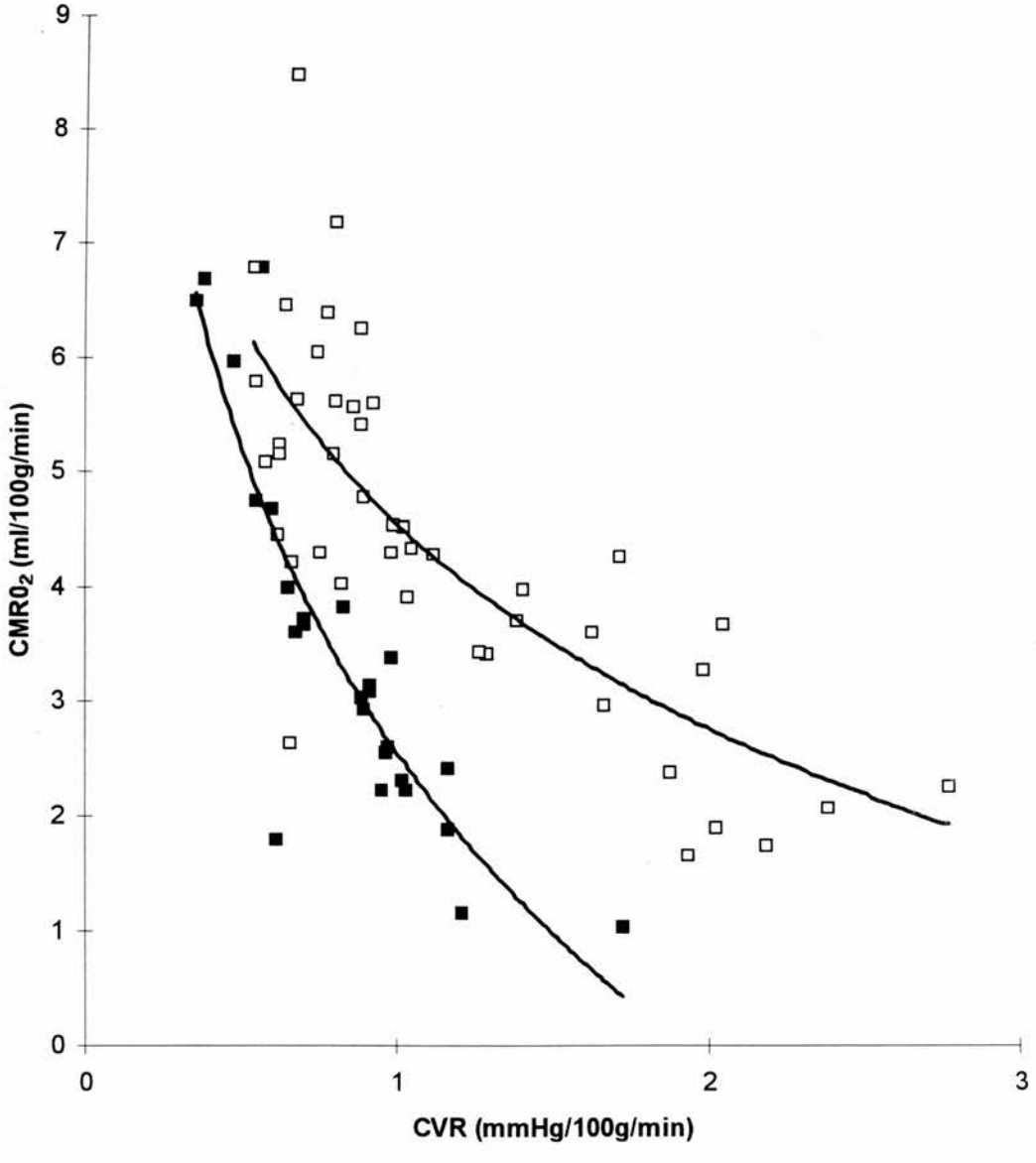


Figure 3.4 - Cerebral metabolic rate for oxygen ($CMRO_2$) plotted against cerebrovascular resistance (CVR). Hyperaemic measurement group (closed squares) ; non-hyperaemic measurement group (open squares).



different curve shifted downwards, indicating a lower level of oxygen extraction for the same vascular tone.

Patient outcome

Glasgow outcome scores were available for 29 patients and were as follows: GOS 1 (dead) - 10 patients ; GOS 2 (vegetative) - 0 patients; GOS 3 (severely disabled) - 10 patients; GOS 4 (moderately disabled) - 5 patients; GOS 5 (good recovery) - 4 patients. Glasgow Outcome Scores were then summarized into two categories - the first representing a good outcome (GOS 4-5) - 9 patients, the second representing a poor outcome (GOS 1-3) - 20 patients.

Looking firstly at the relationship between good or poor outcome and patients categorized as either hyperaemic or non-hyperaemic, we found no significant difference between the hyperaemic and non-hyperaemic patient groups ($p=1.000$). If we examined the relationship between survival or death and hyperaemia or non-hyperaemia, there was again no significant difference ($p=0.414$).

In order to compare good and poor outcome with respect to cerebral blood flow, arterio-jugular venous difference for oxygen and cerebral perfusion pressure, we calculated the mean of each parameter for each patient, because each patient had a variable number of measurements performed. The mean ± SEM cerebral blood flow in the good outcome group was 87 ± 9.5 ml/100g/min compared with 107 ± 11.4 ml/100g/min in the poor outcome group ($p=0.250$). Similarly, the arterio-jugular venous difference for oxygen in the good outcome group was 5.44 ± 0.44 ml/dl compared with 5.06 ± 0.33 ml/dl in the poor outcome group ($p=0.524$). The mean cerebral perfusion pressure was higher in the good outcome group (86.5 ± 3.6

mmHg) than in the poor outcome group (78.9 ± 2.6 mmHg), but this did not achieve statistical significance (p=0.098).

DISCUSSION

The aim of this study was to investigate the adequacy of cerebral haemodynamics in patients with an acute brain injury treated with controlled hypertension. With this approach cerebral perfusion pressure is maintained greater than 70 mmHg, with an arterial pressure orientated approach rather than intracranial pressure treatment directly[166]. A feature of the study was simultaneous determinations of cerebral blood flow and arterio-jugular venous difference for oxygen, to determine the adequacy of cerebral blood flow in relation to metabolic requirement.

Methodological considerations

The nitrous oxide saturation method accurately measures global cerebral blood flow[174], but is unable to detect regional abnormalities of cerebral blood flow that may co-exist in the presence of a normal global cerebral blood flow. However, generalized cerebral blood flow changes have been observed to be superimposed on lesser regional differences by other authors, in both traumatic brain injury and stroke [180 181]. In these studies Xenon[133] cerebral blood flow measurement techniques were used. This allowed the authors to determine that regional and hemispheric differences were of a lesser magnitude than global cerebral blood flow changes.

The Scheinberg modification of the original Kety-Schmidt technique was used in our study. The main difference is the continuous collection of integrated samples over ten minutes instead of drawing ten separate simultaneous samples from the artery and jugular vein. This method is simpler and equally accurate[175]. Diffusion equilibrium between the arterial supply, cerebral tissue and venous nitrous oxide concentration was reached in ten minutes. We considered that the mean difference between the two samples of 4.25% (Table 3.5) was acceptable for a biological measurement and was in accordance with Kety's description of the methodology of cerebral blood flow measurement.

One may question whether ten minutes was sufficient time to achieve complete equilibration of nitrous oxide between the brain and its arterial supply. Kety suggested that if the cerebral blood flow was normal, a study time of ten minutes could result in a 5% overestimation of flow, but if the flow to the white matter was reduced by one half, the overestimation increased to 10 - 12%[182]. However, there is also a risk of underestimating cerebral blood flow by approximately 10%, which relates to the admixture of blood drained from extracranial tissues (the orbital tissue and meninges) and to the slow equilibration of the cerebrospinal fluid with nitrous oxide. Therefore the original nitrous oxide wash-in technique is probably subject to two relatively small errors which are in opposite directions and similar in magnitude. Extending the equilibration time does not necessarily improve the accuracy of the measurement. We found only once that ten minutes was not sufficient time to reach equilibrium (arterio-jugular venous difference for nitrous oxide was greater than 10%) - this measurement was not included in our statistical analysis.

Table 3.5 - Degree of agreement of nitrous oxide measurements. Data are expressed as mean ± SEM.

Sample	Nitrous oxide concentration	
FA	188.5 ± 5.6	(ppm)
FV	180.5 ± 5.5	(ppm)
difference (FA - FV)	4.2 ± 0.4	(%)

Abbreviations:

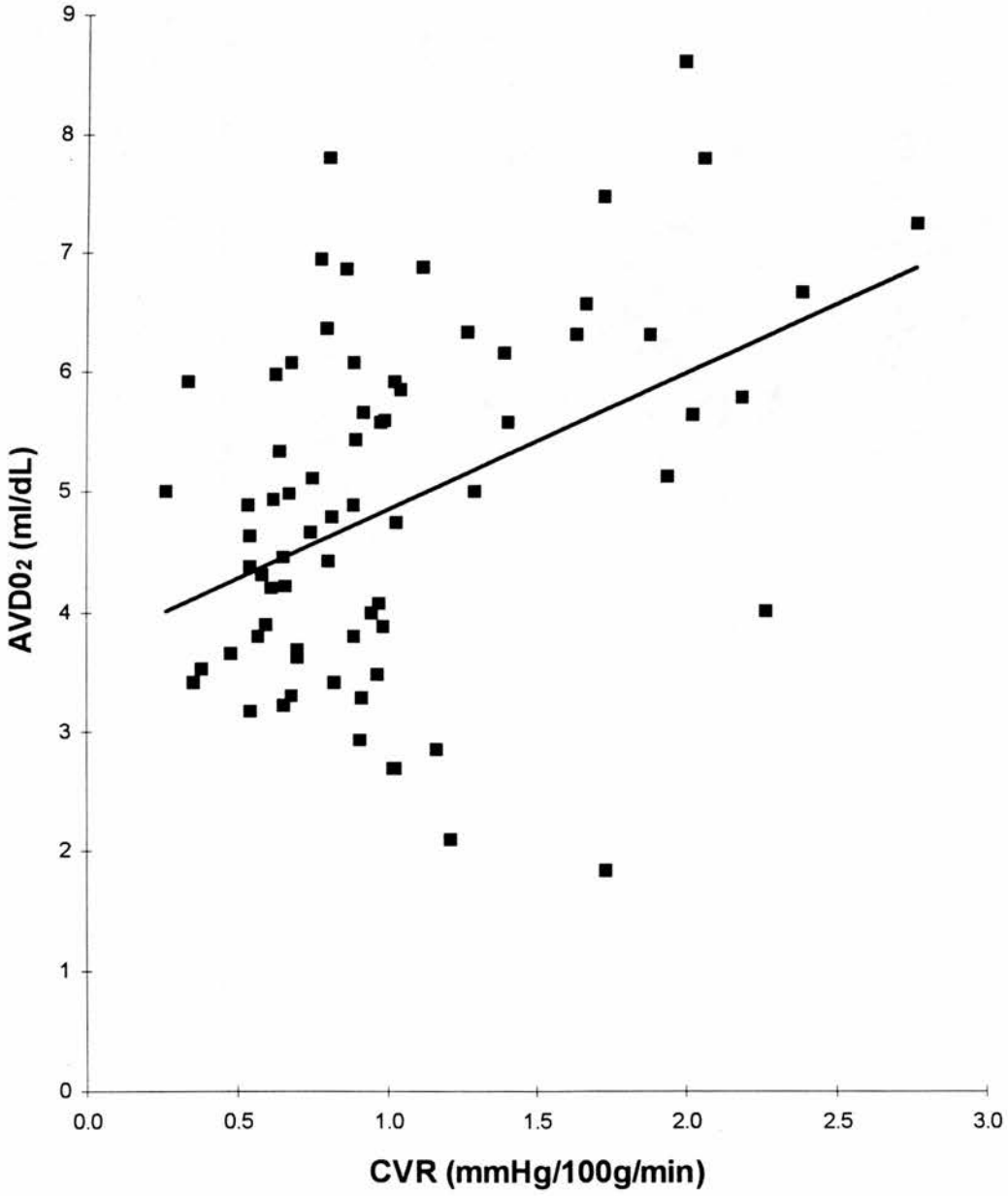
FA = final arterial sample
FV = final venous sample
ppm = parts per million

Cerebral perfusion pressure management and related physiological variables

In our patient population we used a protocol to maintain a cerebral perfusion pressure greater than 70 mmHg, in order to protect the brain from periods of decreased oxygen delivery as a result of arterial hypotension or intracranial hypertension causing a compromised cerebral perfusion pressure. If autoregulation of cerebral blood flow is preserved, an increase in cerebral perfusion pressure within the autoregulatory range will induce cerebral vasoconstriction, causing a decrease in cerebral blood volume and consequently a decrease in intracranial pressure. This mechanism is known as the "vasoconstriction cascade"[166]. We did not assess autoregulation in each patient, but when an increase in intracranial pressure resulted in a reduced cerebral perfusion pressure, the mean arterial pressure was actively increased; this manoeuvre usually caused a reduction in intracranial pressure. To facilitate this increase in mean arterial pressure, euvolaemia was established, then a vasoactive drug infusion (adrenaline, methoxamine or more recently noradrenaline) was started. As a result of this protocol most of the patients had a cerebral perfusion pressure greater than 70 mmHg during the study period.

A interesting finding of this study was the relationship between cerebral blood flow and cerebrovascular resistance, which was described by an exponential equation (Figure 3.3). As cerebrovascular resistance is a parameter calculated from cerebral blood flow, we verified that this relationship was not solely due to "mathematical coupling" by examining the relationship between arterio-jugular venous difference for oxygen and cerebrovascular resistance (both of these variables were measured or calculated independently, Figure 3.5). This showed a positive correlation, indicating that there was indeed a true negative correlation between cerebral blood flow and cerebrovascular resistance. This supports similar findings by Cruz et al.[183]. Under

Figure 3.5 - Arterio-jugular difference for oxygen ($AVDO_2$) plotted against cerebrovascular resistance (CVR). All measurements are shown as solid squares. ($r^2 = 0.17$, $p<0.001$)



physiological conditions, the relationship between cerebral blood flow and cerebrovascular resistance should reflect changes in the activity of vascular smooth muscle. For example if the cerebral perfusion pressure is increased within the autoregulatory range, cerebral blood flow should remain constant, as cerebrovascular resistance should rise. In Figure 3.3 (left-side, closed diamonds), this relationship is absent and the vascular bed behaves passively. This behaviour has been described previously after experimental concussive brain injury[184] and after maximal relaxation of vascular muscle with arterial hypercapnia[185]. In our data-set, the injury may explain the "passive" behaviour of the vascular bed in measurements classified as hyperaemic.

Incidence and significance of hyperaemia

44% of measurements carried out were classified as hyperaemic. Using our categorization for patients, 33% had at least one episode of hyperaemia documented. In a similar population of comatose patients with a traumatic brain injury, Obrist et al.[155] reported at least one incidence of hyperaemia in 55% of patients. Obrist defined hyperaemia with cerebral blood flow measurement criteria (confirmed by arterio-jugular venous difference for oxygen). We defined hyperaemia using the arterio-jugular venous difference for oxygen (< 4 ml/dl), as this highlights the mismatch between oxygen delivery and oxygen demand - in effect a "relative" hyperaemia. This difference in classification may explain the lower incidence of hyperaemia in our population. Our study certainly supports the existence of the "luxury perfusion syndrome" as we have clearly demonstrated cerebral blood flow in excess of metabolic demand.

In the hyperaemic measurement group oxygen extraction was lower than in the non-hyperaemic measurement group, at a similar vascular tone (Figure 3.4); ie the

cerebral metabolic rate for oxygen was lower. This signifies that coupling between blood flow and metabolism persisted and differs from Obrist's study in which there was no relationship between cerebral blood flow and metabolism in hyperaemic patients[155]. There are several possible reasons for this discrepancy. Firstly, we used a different technique to measure cerebral blood flow: Cook et al. showed that nitrous oxide and Xenon methods cannot be directly compared[186]. Consequently, the cerebral blood flow ranges are different. Secondly, we used a different definition for hyperaemia and fewer patients were classified as hyperaemic. Finally, clinical management was not extensively described in Obrist's paper[155]: it is likely to have been substantially different from that of our patients, who, treated with controlled hypertension, probably maintained a higher vascular tone that may have partially restored the "vasoparalysis" described by Obrist.

Transcranial Doppler to define hyperaemia

The mean flow velocity was at the upper limit of the reference range in both the hyperaemic and non-hyperaemic measurements (Table 3.3), which is similar to the results reported by Chan et al.[178], but there was no difference in mean flow velocity between hyperaemic and non-hyperaemic measurement groups. This is not surprising as Chan et al. suggested that the most useful diagnostic parameter for hyperaemia in patients with traumatic brain injury was the absence of the notch in the diastolic component of the Doppler waveform.

Outcome

Outcome results in such a small study population must be viewed with caution, however it was of interest that the cerebral perfusion pressure was marginally

higher in those who progressed to a good neurological outcome than those who had an unfavourable outcome. The non-significant differences seen between hyperaemic and non-hyperaemic patients with respect to outcome should be confirmed in a larger study. A further study should also examine the effects of administration of a vasopressor on outcome. We were unable to analyse this as some patients had vasopressor administered during some measurements, but not others.

Implications for patient management

Our results show that by adopting a management protocol which includes the use of controlled hypertension we are likely to attain an adequate cerebral blood flow and avoid episodes of global brain ischaemia, together with maintaining coupling of cerebral blood flow and metabolism. This approach is also less likely to result in compromised perfusion of other organs than management which focuses solely on treatment of intracranial hypertension with diuretics. In addition, the use of vasopressors did not increase the incidence of hyperaemia reported by other investigators[155], who studied patients who were not receiving such drugs.

While the use of "controlled hypertension" is a relatively simple and inexpensive treatment regime, intensive care units are now being encouraged to purchase and use new technology which forms part of a growing range of sophisticated cerebral monitoring equipment now on the market. Some sources, both commercial and scientific, claim that these monitoring devices are likely to reduce the occurrence of secondary insults to the injured brain, and hence improve patient outcome.

We have already discussed the use of jugular venous catheters in Chapters 2 and 3. These may also be used to monitor jugular venous haemoglobin oxygen saturation. Another method of assessing cerebral oxygenation is near-infrared spectroscopy, a non-invasive technique which is becoming more popular in the intensive care unit.

In Chapter 4 we assess two different monitoring systems.

CHAPTER 4

A COMPARISON OF THE INVOS 3100 AND THE CRITIKON 2020 NEAR-INFRARED SPECTROPHOTOMETERS AS MONITORS OF CEREBRAL OXYGENATION

INTRODUCTION

Near-infrared spectroscopy has been used for many years in neonatal intensive care for the study of intracerebral oxygenation and haemodynamics[187-189], and such monitors are now increasingly used in adult intensive care units and in the operating theatre.

Near infra-red light, in the wavelength region of 700-1000 nm, is poorly absorbed by biological tissue, however oxygen-dependent absorption spectra have been identified in this range for three chromophores: haemoglobin (Hb), oxy-haemoglobin (HbO_2), and cytochrome aa3, the terminal enzyme in the intra-mitochondrial respiratory chain[190]. This absorption is in a manner consistent with the Beer-Lambert law for solutes:

$$OD = \log (Io/I)\text{-}aCl$$

where OD, is the optical density, Io/I is the ratio of the relative intensities of incident and transmitted light, a is the absorption coefficient, C is concentration and l is the optical pathlength. Jöbsis first reported the use of these principles in 1977[191], when he investigated the potential of near infra-red spectroscopy for non-invasive monitoring of tissue oxygenation. He transmitted light through animals' heads, and provided in vivo monitoring of changes in the oxygenation of chromophores in cerebral tissue, describing this as a "biological window" into the brain. At least three wavelengths of near-infrared light need to be used to accurately identify the contributions of the three major chromophores, because the absorption spectra of Hb, HbO_2 and cytochrome aa3 are broad and overlap extensively[192]. The pathlength of light through brain tissue

cannot be measured accurately in vivo, but investigators have estimated it to be around four times the actual distance between the light source and the photodetector. Wray et al. developed empirically derived absorption and scattering coefficients[193], and it has subsequently become possible to produce quantifiable estimations of the percentage concentration of each chromophore by using specific algorithms.

The transmission method is inapplicable in adults because of skull density, but the development of reflectance photometry has solved this problem. As most of the attenuation of near infra-red light in brain tissue is by scattering rather than absorption, light will penetrate a variable degree into the head and be reflected back. The greater the distance from the source to the reflected component, the deeper photons will penetrate. Rather than transilluminating the head, light travels through a banana shaped wedge of tissue. If a second receptor is placed between the emitter and the first receptor, it is possible to correct for the contribution of skin and bone to the derived measurements. The propagation of light in tissues has been mathematically modelled in a variety of ways, ranging from finite element analysis to the "Monte Carlo" or random walk model[194], which offers a better assessment of the anisotropic component of photon diffusion. The potential clinical value of such a noninvasive technique for early detection of cerebral hypoxia, for example intra-operatively or after head injury, has led to the development of several models of adult cerebral oximeters using near infra-red spectroscopy. These can be classified by the type of light source used, ie diffuse or coherent (laser). One of the first was developed by the Hammamatsu Corporation, which used a coherent light source and an array of photomultiplier receptors. It is expensive, difficult to use and demands careful application and removal of extraneous light interference by covering the head. It provides quantifiable measurements and trending of oxy- and deoxyhaemoglobin as

well as the redox state of cytochrome aa3. Two more recently introduced, simpler monitoring systems, the Invos 3100 (Somanetics Corporation, USA)[195] and the Critikon 2020 (Johnson & Johnson Medical, UK) are compared in this study. The former uses a diffuse light source with two photoreceptors contained in a single disposable rubber adhesive patch. The receptors are spaced for subtraction of the subcutaneous signal. It uses an algorithm that eliminates dependence upon the optical pathlength distance. Analysis is made of the content ratios - the relative contribution from oxygenated and deoxygenated haemoglobin. This is dependent upon an assumption of the normal distribution of Hb and HbO_2 in the brain, which is related to the relative volumes of the arterial, venous and capillary circulations. This has been estimated at 70% arterial, 25% venous and 5% capillary, although some researchers have expressed reservations as to the accuracy of this. The end result is a regional haemoglobin oxygen saturation expressed as a percentage, the rSO_2. Early work revealed a positive correlation with SjO_2, and McCormick et al. confirmed that the monitor was able to discriminate between extra- and intracranial circulations with selective perfusion techniques using Indocyananine Green[196]. The Critikon 2020 uses a coherent light source and algorithms which permit quantification of the chromophores Hb, HbO_2 and cytochrome aa3. It has a large, multi-colour display screen and "user-friendly" menu type controls. The sensor is re-usable and is attached to the forehead of the subject with an adhesive pad.

All these monitors may be affected by the presence of extracerebral haematomas and diffuse subarachnoid bleeding, and the validity of measurements in patients with traumatic brain injury requires careful appraisal. Scalp haematomas may also affect readings by altering scalp thickness and hence the photon pathways of the Somanetics and Critikon machines, and so sensor position is important. This effect

has been used to positive ends by the development of apparatus which identifies sites of intracranial haematomas with a movable probe and receptors[197].

In this prospective study, we aimed to compare the performance of the Somanetics 3100 and the Critikon 2020 monitors. The systems were compared in terms of their ability to provide a stable measurement of rSO_2, the variability of that measurement and the agreement between measurements provided by the two monitors, in resting volunteers.

METHODS

Local Ethics Committee approval was obtained in advance. Eighteen healthy volunteers, eight female and ten male, aged between twenty one and thirty years (mean age twenty seven years) were recruited into the study. All gave informed consent to participate. Subjects were seated comfortably and were requested that movement be kept to a minimum during the recording period.

The Somanetics disposable adhesive sensor and the Critikon reusable sensor with disposable adhesive fixation pad were applied to either side of the forehead in accordance with the manufacturers' instructions. The placement sites were free of hair and situated as far above the supraorbital ridge and away from the midline as possible to minimise signal interference from underlying air or venous sinuses. The skin was cleaned and degreased prior to sensor application. A headband supplied by Critikon secured both sensors.

The signals from the oximeters were allowed to stabilise before data collection commenced. The protocol was divided into two stages. The sensors were applied and

steady-state rSO_2 was monitored for at least 30 minutes. At the end of this period the subject was asked to hyperventilate until they experienced a subjective sensation of light-headedness, at which point they should return to their usual breathing pattern. Note was taken of the time of onset and termination of hyperventilation. The sensors were then resited so that each was placed contra-lateral to its original position and the above procedure was repeated. Although the Somanetics sensor is not designed to be reused, its adhesive properties were adequate for this single change in position. Data from both oximeters were logged at intervals of 15 seconds to a personal computer interfaced to the monitors.

Comparisons were made firstly of each monitor's practical ability to provide stable readings of rSO_2 and of their relative performance in male and female subjects. Mean rSO_2 values were calculated for each oximeter for the first 30 minutes of recording. These values were used in a method comparison analysis[198] to assess the agreement (a) between oximeters running simultaneously, and (b) within oximeters sequentially left and right. The mean change in signal observed during hyperventilation was analysed using the same methods in order to compare size and direction of the change in rSO_2 reported. When the bias between paired oximeter readings was not constant over the range of values observed, a correlated regression model was fitted to the bias using the generalised estimating equation (GEE) approach[199]. All analysis was carried out using Splus for Windows 3.2, and the GEE models fitted using add-on functions supplied by V. Carey and A. McDermott of Harvard Medical School.

RESULTS

Ten of the eighteen volunteers participating in the study were successfully monitored for the full hour (eight women, two men, mean age 23 years). In one subject both systems recorded adequately for the first half hour but on relocation of the sensors the Critikon failed to continue monitoring acceptable values. In two cases the Invos performed well, while the Critikon reported a poor or absent signal; in another two, neither oximeter was able to provide reliable readings. Three subjects were abandoned on the basis of Critikon signal problems without assessing the reliability of the Invos measurement. Thus, failure to obtain a stable reading occurred in eight out of eighteen (44%) attempts with the Critikon and in two out of fifteen (13%) with the Invos. The latter was found to work in two instances where the former failed but there were no comparable cases where the Critikon monitored successfully while the Invos did not. In all cases where one or both oximeters were unable to record adequately, the subjects were male; the Critikon showed a significantly higher failure rate in the male participants (Table 4.1, Fisher's exact test, $p=0.001$).

A total of 10.5 hours of data were recorded simultaneously by both monitors from 11 subjects (Table 4.2). A plot of the difference between the oximeter readings against their average value (Figure 4.1) shows a clear trend for the bias to change with the underlying value of rSO_2. For rSO_2 values in the lower range the Critikon tended to read 5 - 10% higher than the Invos, with the reverse true at high saturations. To explore possible causes of this phenomenon a GEE regression model was fitted, which takes account of the fact that we have two correlated measurements from each subject. There was no significant effect upon the bias of either the order of

Table 4.1 - Ability of Critikon 2020 to successfully monitor cerebral oxygen saturation

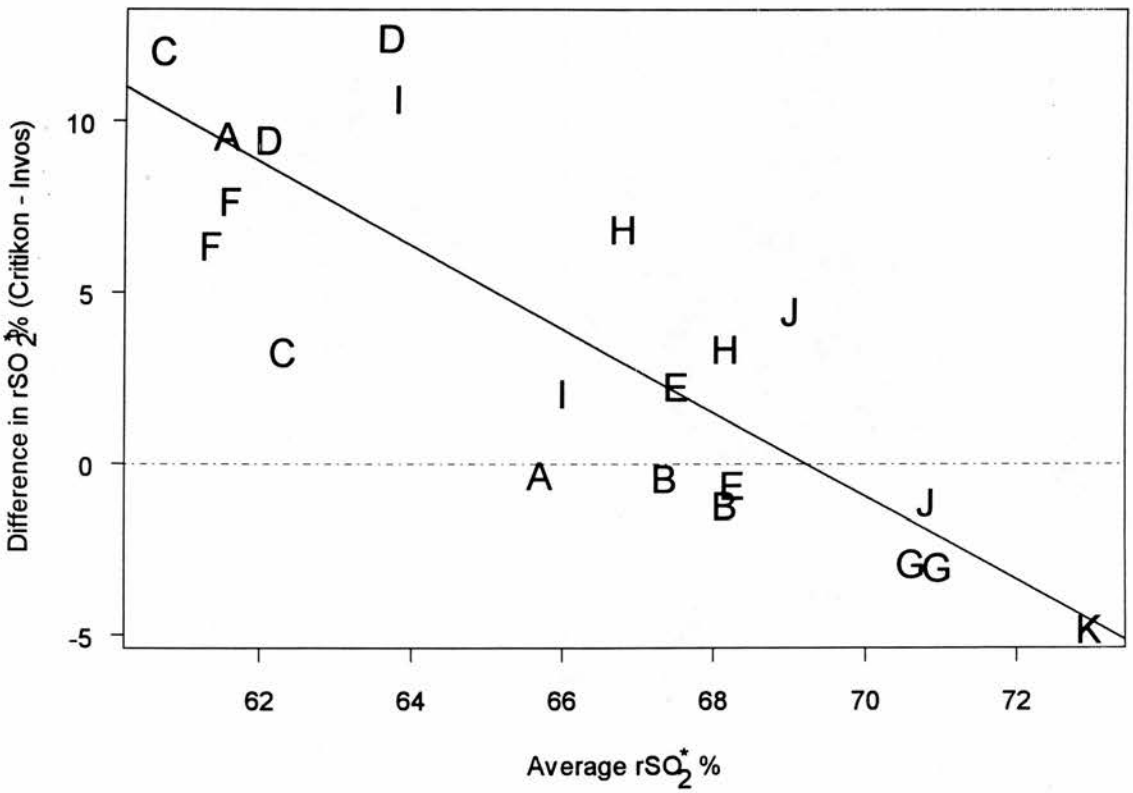
	Critikon successful in monitoring?		
Subjects	Yes	No	Total
Female	8	0	8
Male	2	8	10
Total	10	8	18

Table 4.2- A comparison of baseline readings reported

	Mean rSO_2*at steady state (%)	
	Critikon	**Invos**
Range	64.0 - 71.3	56.7 - 75.3
Mean	68.0	64.3
Standard Deviation	2.1	6.1

* = regional cerebral oxygen saturation

Figure 4.1 - Differences between the regional cerebral oxygen saturation (*rSO_2) shown by the Critikon and Invos monitors, plotted against their average, for each 30 minute period. The letters A to K represent individual subjects.

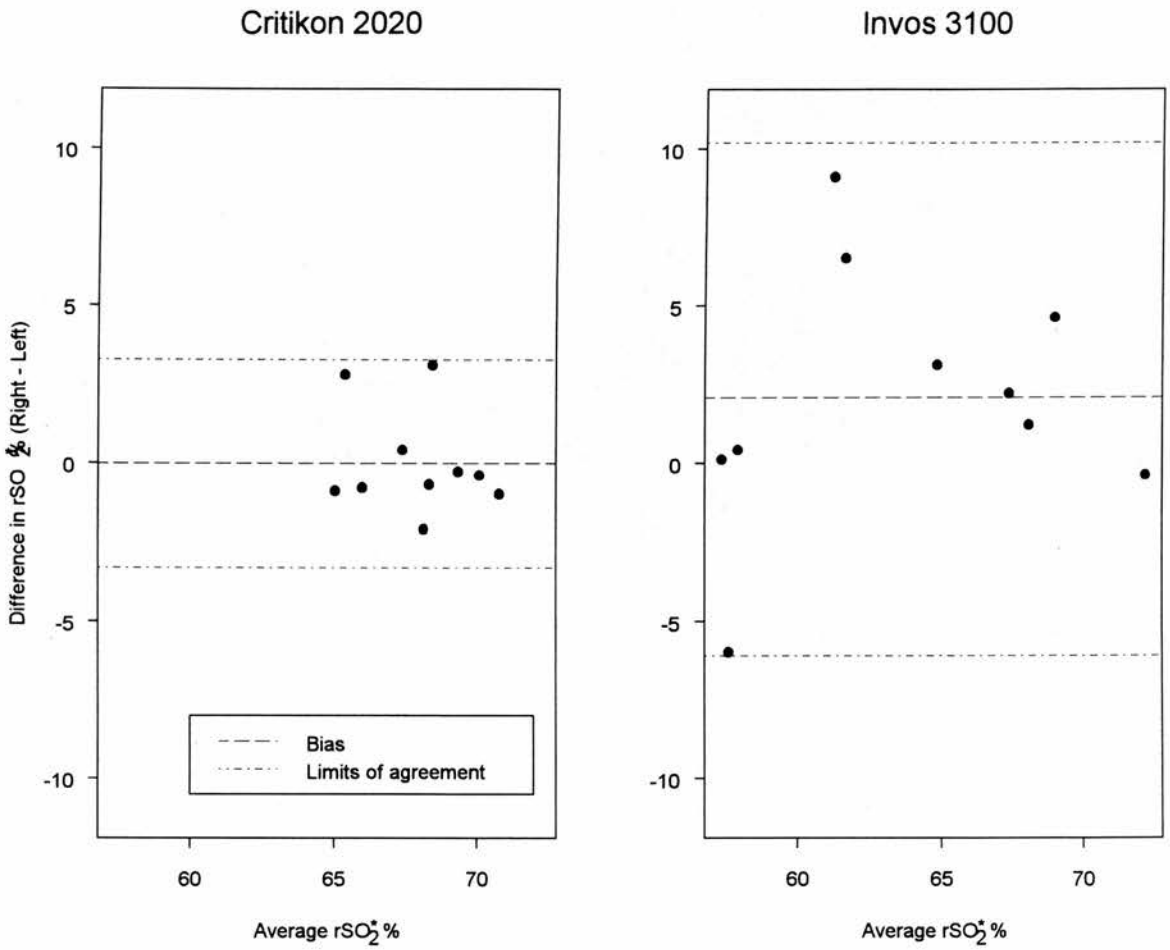


measurement (first 30 minutes versus second 30 minutes, p=0.657) or the location of the oximeters (Critikon left versus Critikon right, p=0.436), but there was a highly significant relationship between the bias and the average rSO_2 (slope=-1.22, p<0.001). The fitted regression line is plotted on Figure 4.1, and shows that from the point of agreement at an rSO_2 of approximately 69%, for each percentage point decrease in rSO_2, we predict the difference between the monitors to increase by 1.22%. The estimated correlation between differences observed within the same subject was 0.133.

For the within-monitor comparisons, there was no apparent relationship between the side-to-side differences and the average value. The observed bias (right - left) for each monitor was not significantly different from zero (Critikon, mean = -0.01, p=0.985 ; Invos, mean = 2.08, p=0.113). The Invos however, did show significantly more variability (F-test for homogeneity, p=0.012), and this is reflected in the limits of agreement, which are (-3.3, 3.3) for the Critikon and (-6.1, 10.2) for the Invos (Figure 4.2).

For the hyperventilation phase of the experiment observed changes in rSO_2 ranged from -4.0% to +1.2% for the Critikon and from -9% to +1% for the Invos. Once again the size of the disagreement between monitors was related to the size of the change ; the larger the average decrease in rSO_2, the larger the difference between monitors. A GEE regression model estimates the point of agreement at approximately -1% and predicts that for every 1% drop in rSO_2 the difference between the monitors (Critikon - Invos) will increase by 1.5% (p<0.001).

Figure 4.2 - Differences between the average regional cerebral oxygen saturation (*rSO_2) shown by each monitor for each 30 minute monitoring period, plotted against their average for the total 60 minutes.



DISCUSSION

Much controversy surrounds the validity of rSO_2 measurements obtained by reflectance cerebral near-infrared spectroscopy[200-202]. The original technique requires transillumination of the neonatal skull, where determination of the optical pathlength (the distance travelled by the photons from emitter to detector) is easily measured. This is not the case in adults, where the use of reflectance spectroscopy means that optical pathlength can only be estimated using complex modelling techniques. The use of a coherent light source, with greater penetrance and less scatter, has theoretical advantages when using these models. In this manner the Critikon 2020, using laser optodes, should be superior to the Invos 3100 which uses a diffuse light source. A further and important difference between the two monitors is the choice of algorithm for quantification of rSO_2. The Critikon uses simultaneous solution of matrix equations of all chromophore absorption, whereas the Invos relies upon a questionable assumption of the compartmentalisation of the cerebral vascular bed.

Our original intention was to conduct this study on ventilated patients in the intensive care unit and was motivated by several factors. Firstly, this technology has been developed for clinical settings such as intensive care where cerebral oxygenation is likely to become compromised and should therefore be assessed within that environment. Secondly, working with this population would have allowed a much longer total monitoring time per subject and minimised the effect of movement artifact, shown to influence near infra-red spectroscopy signals[203]. Finally, we intended to manipulate arterial oxygen saturation and partial pressure of carbon dioxide and correlate these changes with any alteration in rSO_2 reported by each of the oximeters. The insurmountable signal problems experienced with the Critikon instrument forced

a radical revision of the original protocol to be made. It failed to register an adequate signal from any of the four intensive care unit patients to whom it was applied, whilst simultaneous monitoring by the Invos instrument appeared to be consistent and stable. The decision to use healthy subjects was based on the finding that acceptable Critikon readings were only obtainable from this group.

Our results in volunteers demonstrated a higher failure rate with the Critikon in comparison to the Invos. Although the Invos performed poorly in two cases, the Critikon also failed to record. It can be concluded that the Invos system is the more likely of the two to provide a recording that is continuous and stable. The incessant and frustrating 'poor signal' alarm that we experienced with the Critikon is totally unacceptable in a clinical environment.

We observed a strong positive association between failure of the Critikon monitor and male sex. Anatomical features of the head, for example skull thickness and mass of overlying muscle, are known to vary considerably between individuals and between different cranial regions[204 205] which supports the theory that the extent of infrared light scattering also shows inter and intra-subject variability[206]. Adult women tend to have smaller, lighter and thinner skulls than men[205]; the existence of special features including the prominence of the supraorbital ridges and the contours of the forehead which can be used to distinguish between male and female crania[207] is of particular relevance to the present findings. Further support for the observed sex difference is drawn from the suggestion that the amount of light collected by near infra-red spectroscopy at a fixed space is greater for women than for men[206].

Assuming that a similar rSO_2 can be expected in equivalent areas of the two hemispheres in a healthy person, the side-to-side comparisons show that the Critikon monitor, despite the high failure rate, does appear to have significantly less inherent

variability than the Invos. However, the dependence of the disagreement between the monitors upon the underlying value of rSO_2 suggests that either one or both of these machines is not giving a quantified value for regional cerebral oxygen saturation. A difference of up to 10% for values of rSO_2 in the range given by healthy volunteers does not bode well for clinical application of these monitors.

The values of rSO_2 from both monitors decreased during hyperventilation, as was expected because systemic hypocapnia is known to result in cerebral vasoconstriction. Again the disagreement between the monitors varied with the average value, providing further evidence of the uncertainty of these values of rSO_2. However, without objective assessment of the change in arterial blood gas tensions it is difficult to analyse the significance of these changes. In the majority of cases the Invos showed the greater mean decrease in rSO_2, a finding that differs from a previous report in which it failed to respond to an episode of hypercapnia[201]. That study produced more detailed and direct measurements and it therefore seems plausible that the limitations of the present study design may be responsible for any discrepancies.

It is generally accepted that reflectance spectroscopy will become useful as a monitor of tissue oxygenation, and signal changes attributable to variations in cerebral oxygenation have indeed been demonstrated[203 208 209] however further development is required before this technique becomes commonly applied in intensive care. The different algorithms used by the two systems assessed in the present study may explain some of the lack of agreement between them. We strongly suspect that deficiencies in sensor design may explain the unacceptably high failure rate in monitoring attributable to the Critikon instrument.

CHAPTER 5

DISCUSSION

It is some time since Douglas Miller first proposed that "secondary insults" such as hypotension, systemic hypoxia and hyper-pyrexia were important factors associated with outcome after brain injury[162 170]. He suggested that it was not only the severity of the primary injury which was important, but also the progression to secondary injury if patient care was not optimal. His work was instrumental in the introduction of improved intensive care of patients who had suffered a traumatic brain injury[210].

An improvement in intensive care facilities together with the development of specialist neuro-intensive care units has, however, not brought about a dramatic improvement in the numbers of patients who survive a severe acute brain injury with a good neurological outcome. Graham et al. published a paper in 1989 in which neuropathological changes of the brain were studied in patients who had died after a traumatic brain injury[163]. Two time periods were studied: 1968-1972 and 1981-1982. Despite improvements in patient management between these periods, the incidence of ischaemic brain damage was similar in both study periods, at around 90%. Analysis of our own secondary insult database of physiological parameters for patients with an acute brain injury in the intensive care unit reveals a significant reduction in hypotensive and hypoxic insults (ie periods where mean arterial pressure and arterial haemoglobin oxygen saturation fall below acceptable limits) to the brain over a seven year period, but this has not been paralleled by a similar reduction in mortality. We therefore must look more closely at the pathophysiology of brain injury in our attempts to explain why this is the case.

Many "experts" have suggested that secondary injury is caused by an increase in the local concentration of one or more biochemical substances, including the prostaglandins, leukotrienes, superoxides of oxygen or the "excitatory" amino acids.

Results of laboratory work have often excited researchers who have shown that efforts to reduce local concentrations of these substances have resulted in reduced brain tissue damage or improved functional outcome, but clinical trials in humans have invariably been disappointing. This has particularly been the case with a class of drugs known as the NMDA receptor antagonists (results awaiting publication).

Within the past decade there has been an increasing interest in the role of inflammation in brain injury, and in particular the parts played by the cytokines and, more recently, the leucocyte adhesion molecules. Our work in the field of brain inflammation detailed in Chapter 2 showed that there is significant intracranial production of IL-6 after injury, with jugular venous concentrations being significantly higher than arterial. The sources of this cytokine are likely to be both glial and haematological. At present the exact role of IL-6 in the injury process is unclear. Is IL-6 produced as result of secondary injury mediated by other compounds eg IL-1 or the eicosanoids, or is it produced soon after the primary injury, itself leading directly to secondary injury?

Conversely, there is some evidence to suggest that IL-6 may in fact exert protective effects ie it may function as an anti-inflammatory cytokine, and it may be that the high serum concentrations seen in our work, and in the work of others, represent attempts by the immune system to antagonise the effects of the pro-inflammatory cytokines such as IL-1 and TNF.

In addition we found that systemic concentrations of IL-6 were also raised well above normal. This pattern of prolonged elevation of IL-6 is seen in the systemic inflammatory response syndrome and in the adult respiratory distress syndrome (ARDS), and serum concentrations of IL-6 have been shown to be related to outcome in these conditions both in terms of maximum concentrations detected[211] and of

persistence of IL-6 in serum over time[212 213]. The increase in systemic IL-6 seen in our study is unlikely to be the result of intracranial production only; it is likely that there is a systemic inflammatory response after brain injury during which IL-6 is produced by other organs and tissues. These other sources may therefore also be involved in the intracranial inflammatory process. It would certainly be interesting to measure, for example, transpulmonary gradients for IL-6, taking samples from central venous and pulmonary artery catheters in patients with brain injury, and to compare these results with transcranial gradients in each patient. This is one avenue which I may explore in future studies.

There has been little work relating outcome in acute brain injury to IL-6 concentrations in serum. In this study we were unable to show any relationship between these two factors, whereas there was a strong relationship between sICAM-1 concentrations and poor neurological outcome. This suggests that any attempt to antagonise the actions of IL-6 in the clinical setting may not be helpful. There is little if any published work which has explored antagonism of IL-6 in the laboratory, and related this to outcome measures.

Of much greater interest is the theory that antagonism of the pro-inflammatory cytokines IL-1β and TNFα may improve outcome in brain injured patients - but do these cytokines play an important part in the pathophysiology of secondary brain injury in humans? Unfortunately I was unable to show any increase in the serum concentrations of these cytokines in my patient group. This reason for this is likely to be that as a tertiary referral centre, we receive these patients some time after the injury has occurred - in this study a median time of eight hours thirty minutes after injury. The peak of production of these pro-inflammatory cytokines is probably within 2-4 hours of injury, and with a half life of not more than 30 minutes, they would have

disappeared from the circulation before I was able to take the first serum samples. Hence another possibility for future work is to gain earlier access to the patient to sample systemic blood. One opportunity may be to work with neurosurgeons at the Royal London Hospital who are involved in the Helicopter Emergency Medical Service. Here medical staff have early access to a substantial number of patients who have sustained a closed head injury.

I was, however, able to show a strong link between sICAM-1 concentrations and poor neurological outcome. Upregulation of the production of adhesion molecules occurs later than the peak activity of IL-1β and TNFα, and is therefore more open to antagonism at the point when the patient is admitted to the intensive care unit. The work carried out with adhesion molecule antagonists in animal stroke models by the two American groups discussed in Chapter 1 is of great interest[124-127 130]. The next step must be to extend this work to look at rats which have sustained a traumatic brain injury, and I believe that there is a German group which has started such a project. The results of the phase 3 trial underway in stroke patients at present will also be a very important pointer as to the likely success of this therapy in other types of acute brain injury.

The fact that we were unable to show any transcranial gradient for the adhesion molecules is perhaps a little surprising given the strong relationship between sICAM-1 concentrations and both neurological outcome and severity of neurological injury. One might imagine that significant upregulation of ICAM-1 production in cerebral micro-vessels would lead to increased concentrations of sICAM-1 in jugular venous blood. Again we must look to the fact that both local intracranial and systemic upregulation of adhesion molecule activity is likely to be important. As I have

discussed above with reference to IL-6, it would be interesting to examine concentrations of adhesion molecules in blood draining other organs in the body.

Our findings of a marked reduction in serum sL-selectin contrast with the majority of studies which have looked at this adhesion molecule in other disease states. One important study however, published by Donnelly et al[111], found a similar reduction in sL-selectin in patients thought to be at risk of developing ARDS. Reduced concentrations of sL-selectin in serum correlated with the development of ARDS and poor outcome. Similarly, in Fassbender's study[110], reduced serum sL-selectin was found in stroke patients.

There is no previously published clinical work which has studied serial concentrations of adhesion molecules in traumatic brain injury and subarachnoid haemorrhage in either arterial or jugular venous serum, therefore my work must be regarded as merely the first step in the investigative process. A study by Fassbender et al.[110] looked at serial changes of adhesion molecules after acute ischaemic stroke and found a rise in sICAM-1 and a reduction in sL-selectin. Jander et al found increased sICAM-1 in the serum of patients with bacterial meningitis[106], but not other neurological diseases, such as multiple sclerosis and the Guillan-Barré syndrome.

Measurement of systemic sICAM-1 concentrations may be helpful in prediction of those cases which should do well, and those which are likely to die or do badly. Although it would be impossible to make a prediction of neurological outcome with any degree of certainty, the assay may be able to assist clinicians in decision making, particularly if repeated over the first 2 days of admission. The development of microdialysis catheters which allow frequent sampling of brain tissue fluid may also allow our research group to improve our knowledge of the time course of inflammatory mediators in brain tissue itself. Further laboratory work must also

include immunohistochemical analysis of brain tissue specimens, both from "live" brain taken during craniotomy and from post-mortem specimens, to examine adhesion molecule activity in micro-vessels and brain tissue.

My work has also given further support to the theory that both S-100 and NSE are useful markers of brain injury. Measurement of these compounds in serum may also help to predict outcome in individual cases. Microdialysis may in future offer a suitable vehicle for ongoing frequent analysis of these molecules, which may give the intensivist additional information to aid further management.

Much of the work summarised in chapter 2 deals with the future, whereas the novel therapy discussed in Chapter 3 is happening now in many intensive care units across the world, without any randomised controlled trials having been carried out to suggest that it improves outcome. Whilst many, led by Michael Rosner[166], believe that induced hypertension should be part of "standard" management for patients with traumatic brain injury, there is another body of opinion which advocates the opposite! A very different approach is adopted by others, led by "the Lund group" from Sweden[214-216]. They argue that to avoid cerebral oedema, patients with traumatic brain injury should be treated with cerebral vasoconstrictors and hypotensive agents. Both groups claim to have similarly good outcome figures, but the studies are small and are certainly not controlled studies of one treatment versus the other. The majority of intensivists agree with the Rosner approach, but some believe that in some cases where cerebral autoregulation is deficient, induced hypertension may cause intracranial hypertension and therefore worsen cerebral ischaemia.

In Chapter 3 we showed that every measurement of cerebral blood flow made in our patient group was either within or above the normal range. Global cerebral hypoperfusion was therefore not detected during our study. There is therefore a

discrepancy between our results and the common finding at post-mortem of ischaemic brain damage in fatal traumatic brain injury as discussed at the start of this chapter[163].

Possible explanations include the effects of our clinical management or the limitations of the cerebral blood flow measurement technique. All the methods based on the Fick principle express cerebral blood flow in terms of ml/100g of brain tissue perfused per minute. If there are areas which are not perfused, they will not be included in the calculation. Therefore, even in the absence of global ischaemia, there may be areas of the brain where blood flow is reduced to critical levels and which may co-exist with areas of hyperaemia. Heterogeneity of cerebral blood flow cannot be measured by arterio-jugular venous differences across the whole brain. Another possibility is that global cerebral hypoperfusion was not detected because cerebral blood flow was measured only when the cerebral perfusion pressure was stable, but this method was employed to avoid interfering with the clinical management of patients and to obtain reliable cerebral blood flow measures.

Many researchers have suggested that cerebral ischaemia, if present, occurs early after injury and may have resolved by the time cerebral blood flow is measured in the intensive care unit. Our measurements were all performed several hours or days after the injury, and it may be that we missed periods where cerebral blood flow was compromised. Bouma et al. reported that periods of low blood flow were common in the first few hours after injury - a cerebral blood flow below the threshold for infarction (18 ml/100g/min) was present in 30% of measurements obtained within the first 6 hours after trauma - and that flow rapidly increased in subsequent measurements[217]. They also found that periods of low cerebral blood flow which occurred at more than 24 hours after injury were associated with a low arterio-jugular venous difference for oxygen, suggesting a low cerebral metabolic rate rather than

frank ischaemia. In our study we showed that in many cases, cerebral blood flow was in excess of metabolic requirements (hyperaemia) and that arterio-jugular difference for oxygen was reduced. It may therefore be that, as all our measurements were carried out at least 6 hours after brain injury, we missed periods of reduced cerebral blood flow, in a fashion similar to our missing the peak production of the cytokines IL-1β and TNFα.

The absence of global ischaemia by blood flow criteria is backed up in our study by the absence of any ischaemia both by arterio-jugular difference for oxygen and lactate oxygen index criteria. Taken together these results form a convincing argument against the theory that, as autoregulation is often absent in those who have sustained a traumatic brain injury, induced hypertension will often result in cerebral ischaemia. Indeed it is also interesting to note that the average mean arterial pressure was actually higher in the group of patients who had had no episodes of hyperaemia noted at all.

What do these results mean in practical terms? Obviously without a properly conducted randomised controlled trial into hypertension / normotension / hypotension as a mainstay of treatment for traumatic brain injury, I am unable to advocate one method with any authority. It is now unlikely that such a trial will ever be carried out, as many would find it unethical to treat patients in a "hypotensive" group in such a study. This is a good example of a problem which has been highlighted by one of the statisticians (David Signorini) working in brain injury at several international meetings recently - that is the lack of any good Class 1 evidence to support many of the treatment and monitoring practices currently employed by those neurosurgeons and intensivists managing patients who have sustained an acute brain injury.

My study, however, suggests that complications resulting from the use of induced hypertension are unlikely, and that it is not a dangerous therapeutic approach. Indeed it is now almost universally employed in the management of spontaneous subarachnoid haemorrhage, and I believe will in future become part of standard management in traumatic brain injury.

The use of controlled hypertension to increase cerebral perfusion pressure is directed towards maintaining or increasing oxygen delivery to brain tissue. Technological developments in monitoring of cerebral oxygenation mean that continuous jugular venous haemoglobin oxygen saturation monitoring is employed in many neuro-intensive care units - indeed it is standard practice in our unit in Edinburgh. This means that periods during which arterio-jugular venous oxygen content difference is increased - suggesting that oxygen delivery to the injured brain is inadequate - are now less common and less prolonged, as they are treated immediately. Invasive probes which monitor the partial pressure of oxygen in brain tissue directly are now in clinical use[218-220]. Again these can provide an early indication that oxygen delivery to brain tissue is inadequate.

As I have discussed in Chapter 4, near-infrared spectroscopy is likely to be an important monitoring technique in the future. It is non invasive and therefore easily applied in all intensive care units, particularly in those outwith neurosurgical centres. There will of course be those who feel that the "explosion" in monitoring technology is perhaps of more benefit to the companies which manufacture these often hugely expensive items of equipment, than to patients (I showed in Chapter 4 that the Critikon 2020 was in effect useless in the intensive care unit). Again ideally we should carry out large randomised controlled trials which would firmly establish whether an item of monitoring resulted in improved outcome. Practically of course this is just not

possible. There would be the ethical problem of treating patients without monitoring that some would regard as essential. There would also be the problem of standardising treatment so that outcome would not be biased by other differences in management.

At present the near infrared spectroscopy monitors are inadequate for routine use on patients with brain injury, but it will not be long before the technical difficulties experienced so far are overcome, and despite the reservations of some intensivists, they are commonly used in both the operating theatre and intensive care unit to monitor cerebral oxygenation.

Finally I need to bring my ideas together. I have mentioned the improved monitoring now available, and at a novel therapy which is now routine in our intensive care unit, however if I again look back at Chapter 3 and examine our outcome figures it is apparent that neurological outcome was disappointing with only nine out of twenty nine patients having a good outcome. These figures are not atypical for a similar group of patients in other neurointensive care units.

As a result of improvements in management and monitoring, oxygen delivery to the brain, except perhaps during short periods soon after injury, is likely to be maintained in the intensive care environment, in the absence of uncontrollable intracranial hypertension. This therefore raises a fundamental question that can lead to much speculative discussion: *why if oxygen delivery to the injured brain is equal to, or in many cases well above the metabolic requirements of the brain, do we see ischaemic brain damage so frequently after brain injury?*

I believe that we must look to failure of cellular oxygen consumption as a possible cause of ischaemic cell damage, ie a state of histotoxic hypoxia exists. This is not a new suggestion. As long ago as the mid 1980s workers such as Yang et al.[221] and Vink et al.[222] proposed that traumatic brain injury resulted in an impairment of

brain mitochondrial respiratory chain function, which meant that oxygen delivered to brain tissue could not be used for aerobic metabolism. There is substantial evidence from experimental work that mitochondrial function is abnormal in animal models of cerebral ischaemia[223 224], and in some studies these abnormalities appear to worsen as time progresses[224]. Poor neurological outcome has also been correlated with persisting abnormalities of mitochondrial function[224 225].

We can therefore speculate that in certain inflammatory disease states, production of pro-inflammatory mediators may prevent cells from utilising the oxygen supplied to tissues. This may explain, for example, why therapeutic efforts directed at increasing oxygen delivery to tissues have not resulted in improved outcome in the systemic inflammatory response syndrome.[226 227]. After an initial primary cerebral injury, with transient ischaemia followed by reperfusion, an inflammatory response is initiated. Mediators such as the cytokines, arachidonic acid metabolites and oxygen free-radicals cause microvascular dysfunction, with plugging of small vessels by platelets and neutrophils, and migration of neutrophils into brain tissue. This is associated with mitochondrial calcium overload with a reduction in their respiratory capacity[228 229]. The worsening of mitochondrial function with time may be associated with a gradual increase in the activity of pro-inflammatory mediators, such as ICAM-1, in patients who go on to a poor neurological outcome.

There would appear to be no direct chain of events which can be easily broken at one point, but a complex web of inter-related processes and mediators which ultimately leads to cell death. To improve outcome after acute brain injury we need to prevent the processes which lead to cell death from occurring. Improved monitoring techniques and careful attention to the metabolic requirements of the brain whilst in the intensive care unit are important, but new drug therapies which antagonise the

effects of the inflammatory process after brain injury may, in the future, prove to be a significant step forward in the management of this devastating condition.

CHAPTER 6

PUBLICATIONS, PRESENTATIONS AND ACKNOWLEDGEMENTS

PUBLICATIONS

Mascia L, McKeating EG, Andrews PJD. Cerebral blood flow and metabolic rate for oxygen following acute brain injury, managed with induced arterial hypertension. *British Journal of Anaesthesia* 1996: 77, 285-286p

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PRINTED ABSTRACTS / PRESENTATIONS

McKeating EG, Mascia L, Signorini DF, Andrews PJD. A comparison of transcranial interleukin 6 gradients following traumatic brain injury and subarachnoid haemorrhage. *Proceedings of the International Congress on Recent Advances in Neurotraumatology* (ICRAN), Rimini, Italy 1996, p465

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CHAPTER 7

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APPENDIX

PUBLISHED PAPERS

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Transcranial cytokine gradients in patients requiring intensive care after acute brain injury

E. G. McKeating, P. J. D. Andrews, D. F. Signorini and L. Mascia

Summary

After acute brain injury there may be increased intracranial production of cytokines, with activation of inflammatory cascades. We have sought to determine if a transcranial cytokine gradient was demonstrable in paired sera of 32 patients requiring intensive care after acute brain injury. The difference between concentrations of IL-1β, IL-6, IL-8 and TNFα in jugular venous and arterial serum was measured on admission, and at 24, 48 and 96 h after the primary injury. There were no differences in IL-1β, IL-8 or TNFα, but median gradients of 6.7 and 11.5 pg ml^{-1} for IL-6 were demonstrated in the traumatic brain injury (n=22) and subarachnoid haemorrhage (n=10) groups, respectively (normal values in serum <4.7 pg ml^{-1}; P<0.001 both groups). This suggests that there is significant production of IL-6 by intracranial cells after acute brain injury. Therapy directed towards combatting the negative effects of IL-6 may potentially benefit patients who have sustained an acute brain injury. (*Br. J. Anaesth.* 1997; **78**: 520–523).

Key words
Brain, injury. Polypeptides, cytokines. Intensive care.

The cytokines are a variety of polypeptide molecules which function as mediators within the communication network of the immune system.[1] They have been implicated in the pathophysiology of many disease processes, including the systemic inflammatory response syndrome (SIRS),[2] acute respiratory distress syndrome (ARDS)[3] and multiple trauma.[4] Many of the biological effects of the cytokines are clinically evident after acute brain injury; these include neutrophilia, pyrexia and alteration of endothelial permeability,[5] which may result in cerebral oedema,[6] as a result of disruption of blood–brain barrier function.

Increased concentrations of serum tumour necrosis factor-α (TNFα),[7] interleukin-6 (IL-6)[8] and intraventricular fluid IL-6 and IL-1β[9] have been demonstrated after traumatic brain injury, and experimental work has demonstrated that glial cells are able to manufacture cytokines.[10 11] After acute brain injury it has been suggested that increased intracranial production of pro-inflammatory cytokines, with consequent induction of auto-destructive inflammatory cascades, results in secondary injury to the brain, causing altered brain metabolism and cell death.[12] With the increased use of intra-parenchymal solid state methods for monitoring intracranial pressure, access to cerebrospinal fluid for analysis is often not possible, and where it is, sampling from catheters may result in ventriculitis.[13] Insertion of a fibreoptic catheter into the jugular bulb to monitor jugular venous haemoglobin oxygen saturation is now standard practice within our intensive care unit (ICU) in acute brain injured patients, giving ready access to blood draining from the brain.[14] We investigated the hypothesis that systemic concentrations of the cytokines IL-1β, IL-6, IL-8 and TNFα are increased after acute brain injury, and that increased intracranial production of these cytokines results in a demonstrable transcranial cytokine gradient, with jugular venous concentrations higher than arterial concentrations.

Patients and methods

After obtaining local Ethics Committee approval, we studied 32 patients who had sustained an acute brain injury (traumatic brain injury (TBI) or spontaneous subarachnoid haemorrhage (SAH)) requiring intensive care. Patient data consisting of sex, age and Glasgow coma score (GCS) after non-surgical resuscitation were recorded on admission to the ICU (see table 1). One patient whose initial GCS was recorded as 15 was anaesthetized at the scene soon after injury because of flail chest. Computerized tomography (CT) subsequently demonstrated severe traumatic brain injury.

A dual-lumen Edslab 4-French gauge oximetry catheter (Baxter Healthcare Corporation, Irvine, CA, USA) was inserted into the jugular bulb on the dominant side of the cerebral venous drainage, as described previously.[15 16] Satisfactory positioning was ensured by lateral x-ray of the cervical spine (catheter tip seen cephalad to the upper border of the C2 vertebral body), and the correct catheter distance marking (e.g. 15 cm) showing at the valve of the sheath was written in the notes for future

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Table 1 Characteristics of patient population (median (range)). The characteristics of the subset, including only the first 20 patients, are shown in parentheses

	TBI group (n=22 (12))	SAH group (n=10 (8))
Sex (M/F)	18/4 (9/3)	6/4 (4/4)
Age (yr)	34 (35)	52 (51.5)
	17–69 (17–49)	21–65 (21–65)
GCS	7 (7)	7 (7)
	3–15 (3–15)	5–10 (5–10)

Table 2 Performance characteristics of each assay. To assess intra-assay precision, three samples of known concentration were assayed 20 times on one plate. To assess inter-assay precision, three samples of known concentration were assayed in 20 separate assays

	Intra-assay coefficient of variation (%)			Inter-assay coefficient of variation (%)		
Sample	1	2	3	1	2	3
IL-1β	3.4	4.4	2.8	8.4	4.2	4.1
IL-6	4.3	1.7	2.1	6.3	3.3	7.2
IL-8	3.9	2.4	3.3	12.2	9.1	7.3
TNFα	5.2	4.2	4.6	7.4	4.6	5.4

reference. An intra-arterial catheter was inserted (if not already *in situ*), usually in the radial artery, as is our standard practice.

Paired arterial and jugular venous blood samples were obtained at designated times after brain injury: within 12 h (median 8 h 30 min, range 2–14 h), and at 24, 48 and 96 h. Time of brain injury in the SAH group was noted as the time of sudden deterioration in GCS by 3 points or more. Patients who did not exhibit sudden neurological deterioration were not included. The diagnostic criterion for SAH was the presence of subarachnoid blood on the CT scan. Satisfactory positioning of the jugular bulb catheter was ensured before each sample was obtained by checking that the correct distance marker of the catheter was showing at the sheath valve. Samples were allowed to clot at room temperature, spun down in a centrifuge at 4000 rpm for 10 min and the supernatant removed and frozen immediately at −25°C. Analysis of serum for IL-1β, IL-6, IL-8 and TNFα was performed by enzyme-linked immunosorbent assay (Quantikine Human Cytokine Assays, R&D Systems) according to the manufacturer's instructions. Inter- and intra-assay coefficients of variation for each assay are shown in table 2. All analyses were carried out by a single operator using the same equipment and procedures throughout. Repeat freeze–thaw cycles for serum samples were avoided by separating the serum from each patient into four tubes before freezing.

A total of 138 samples (69 pairs) were analysed in duplicate for each cytokine (with an additional 80 samples for IL-6) and results averaged to give the final concentration. Samples which gave a result higher than the highest standard supplied with the kit were diluted and the assay repeated. Sera from eight healthy volunteers were analysed as controls. Standard curves and cytokine concentrations were calculated on a personal computer interfaced to a micro-plate reader using dedicated software (Bio-Rad Laboratories). Statistical analysis was by Wilcoxon rank sum and signed rank tests using Splus for Windows 3.2.

Results

In 119 of the 138 samples assayed for IL-1β (86%), concentrations were undetectable (<3.9 pg ml^{-1}). The highest concentration in the remaining 19 (14%) was 7.0 pg ml^{-1}. For TNFα, in 122 samples (88%) concentrations were undetectable (<15.6 pg ml^{-1}). The highest concentration in the remaining 16 (12%) was 31 pg ml^{-1}. IL-8 was detected in 72 samples (52%) (lower limit of detection 94 pg ml^{-1}), but only 10 (7%) showed concentrations >300 pg ml^{-1}. There was no difference between jugular venous and arterial concentrations of IL-1β, IL-8 or TNFα in those patients

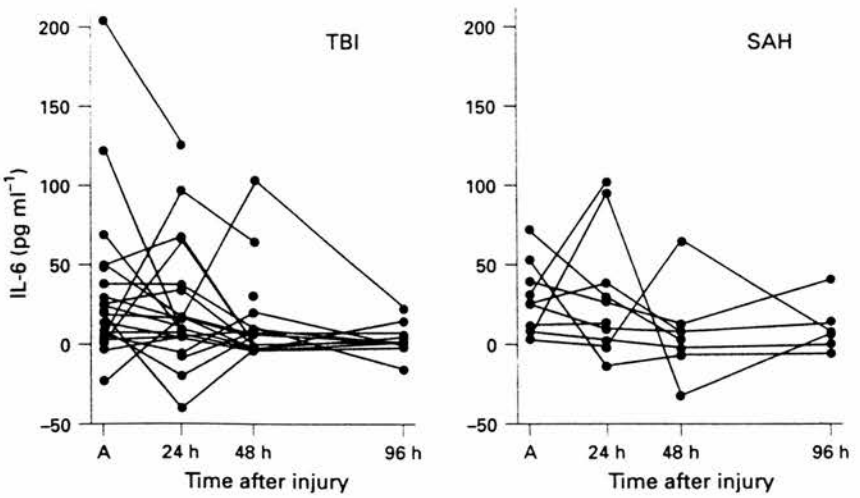

Figure 1 Differences between jugular venous and arterial concentrations of IL-6 in traumatic brain injury (TBI, n=22) and spontaneous subarachnoid haemorrhage (SAH, n=10), over a 4-day period after injury (A=admission). Data may be incomplete because of patient death, discharge from ICU or values lying outside ELISA quantification values. For the TBI group, at each time, n=20, 21, 19 and 11, respectively; for the SAH group, n=10, 10, 8 and 6, respectively.

with detectable concentrations. Control samples all showed undetectable concentrations.

IL-6 was detected in all 218 samples. In the controls, concentrations were below detectable limits (<3.1 pg ml^{-1}) in four samples. The highest concentration in the remaining four control samples was 4.7 pg ml^{-1}. In four pairs of patient samples (3.7%), both samples showed concentrations >1500 pg ml^{-1} (the upper limit of quantification) and were excluded from statistical analysis. In 82 (78.1%) of the remaining 105 pairs, jugular venous concentration was higher than arterial. In the TBI group, median jugular venous concentration was 74.8 (range 19.9–539.8) pg ml^{-1}), median arterial concentration was 63.5 (11.4–518.4) pg ml^{-1} and the median difference was 6.7 (−40.8 to 244.4) pg ml^{-1} ($P<0.001$). In the SAH group, median jugular venous concentration was 64.6 (19.1–455.4) pg ml^{-1}, median arterial concentration was 54.1 (12.2 to 407.4) pg ml^{-1} and the median difference was 11.3 (−32 to 135.2) pg ml^{-1} ($P<0.001$).

Figure 1 shows the individual patient profiles for transcranial IL-6 gradient for each group. In the TBI group, median gradients at each time point were 19.2 ($P<0.001$), 10.5 ($P=0.014$), 6.3 ($P=0.018$) and 1.6 pg ml^{-1} ($P=0.147$), respectively. In the SAH group, median gradients were 26.8 ($P=0.002$), 20.1 ($P=0.027$), 5.6 ($P=0.383$) and 7.8 pg ml^{-1} ($P=0.094$), respectively. Comparing the TBI and SAH groups, there was a significant difference in age between the groups ($P=0.028$), but there was no difference in GCS ($P=0.979$). There was no overall difference in IL-6 gradients across the brain between the TBI and SAH groups ($P=0.350$); there were no differences between groups at any of the four sampling times ($P=0.559$, 0.627, 0.832, 0.366, respectively).

Discussion

We were unable to derive any useful information from measurement of either arterial or jugular venous serum concentrations of IL-1β, IL-8 or TNFα at these times after acute brain injury. It is generally believed that IL-1β and TNFα are released in the early post-injury phase,[17,18] resulting in increased synthesis of IL-6, in addition to other mediators. IL-6 may act to decrease production of IL-1β and TNFα via a negative feedback mechanism.[19] It may be that we missed an early peak of production of IL-1β and TNFα before the patients reached the ICU. The relatively high lower limit of detection for IL-8 (94 pg ml^{-1}) in this particular assay may have masked any significant transcranial gradient.

Results for IL-6 show increased jugular venous concentrations of this cytokine relative to arterial concentrations, particularly within 48 h of brain injury. Transcranial gradients in the TBI and SAH groups were similar, despite different aetiologies of primary injury, suggesting that the mechanisms of the inflammatory process may be similar. The median transcranial gradient on admission in each group was 5–6 times higher than the highest systemic concentration measured in the controls. This suggests significant intracranial production of IL-6. The source of this increased production is likely to be glial cells, and there is experimental evidence to suggest that both astrocytes[10] and microglial cells[11] may be involved. In addition, systemic concentrations of IL-6 were increased greatly. This may be caused solely by intracranial production, but is much more likely to be a result of release of IL-6 from extracranial sources, that is a mild SIRS. Variation in systemic concentrations of IL-6, jugular venous–arterial differences, and different patterns of concentration gradients seen over time may be related to the severity of intracranial injury, type of injury, presence of extracranial injuries or secondary insults to the brain while in the ICU.

This pattern of prolonged elevation of IL-6 is seen in SIRS and ARDS, and serum concentrations of IL-6 have been shown to be related to outcome in these conditions, both in terms of maximum concentrations detected[20] and persistence of IL-6 in serum over time.[21,22] There has been little work relating outcome in acute brain injury to IL-6 concentrations in serum. We are presently collecting outcome data for our group of patients.

A study which compared post-mortem appearances of the brains of non-survivors of traumatic brain injury in Glasgow over two periods (1968–1972 and 1981–1982) showed that the incidence of ischaemic damage had not changed, despite improved intensive care in this group of patients.[23] Analysis of our own secondary insult database of physiological variables for acute brain injured patients in the ICU revealed a significant reduction in hypotensive and hypoxic insults (i.e. periods where mean arterial pressure and arterial haemoglobin oxygen saturation decreased to below acceptable limits) to the brain over a 7-yr period, but this has not been parallelled by a similar reduction in mortality. We can speculate that in inflammatory disease states the mediators produced may prevent cells from using oxygen supplied to tissues, that is there exists a state of histotoxic hypoxia. This may explain why therapeutic efforts directed at increasing oxygen delivery to tissues have not resulted in improved outcome in SIRS.[24,25] If this hypothesis is correct then a different therapeutic approach is necessary.

Future management of acute brain injured patients may include pharmacological modification of the inflammatory processes involved in the pathophysiology of the condition, using antibodies or receptor antagonists, preventing further cell death and improving outcome.

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APPARATUS

A comparison of the Invos 3100 and the Critikon 2020 near-infrared spectrophotometers as monitors of cerebral oxygenation

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Summary

Assessment of cerebral oxygenation using near-infrared spectroscopy in intensive care is increasing. We compared the ability of the Invos 3100 and the Critikon 2020 monitors to produce stable and consistent readings of regional cerebral oxygen saturation in resting volunteers. Failure to obtain any stable reading with the Critikon occurred in eight out of 18 subjects (44.4%) and with the Invos in three out of 15 subjects (20%). The Critikon showed a significantly higher failure rate in male subjects ($p = 0.0011$). Differences in recorded values of cerebral oxygen saturation (Critikon – Invos) ranged from −4.7% to 12.6% and were significantly related to the average saturation level ($p < 0.0001$). The within-monitor variability was significantly higher for the Invos ($p = 0.0124$). Neither monitor is able to give stable and consistent readings over time, particularly in male subjects. The unacceptably high failure rate of the recently introduced Critikon 2020 will limit or prevent its clinical use.

Keywords *Equipment*; near-infrared spectrophotometer. *Brain*; oxygenation.

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Near-infrared spectroscopy (NIRS), introduced in 1977 by Jobsis [1], has been used for several years in neonatal intensive care for the study of intracerebral oxygenation and haemodynamics [2–4]. The technique relies upon the relative transparency of soft biological tissues (including the fetal skull) to radiation in the near-infrared region (700–1000 nm) and upon the presence in tissues of chromophores whose light-absorbing properties vary with oxygenation. In cerebral tissue these chromophores are oxyhaemoglobin, deoxyhaemoglobin and cytochrome oxidase aa3, the terminal enzyme of the mitochondrial respiratory chain. This transmission method is inapplicable in adults owing to skull density, but a reflectance technique has been developed which purports to measure absorption in the cerebral cortex. After the application of subtraction algorithms to remove extracranial contributions, estimates of regional percentage haemoglobin oxygen saturation (cerebral oxygen saturation or rSO_2) can be derived from the signals.

The potential clinical values of such a noninvasive technique for early detection of cerebral hypoxia, for example intra-operatively or following head injury, has led to the development of several models of adult cerebral oximeters using NIRS. These can be classified by the type of light source used, i.e. diffuse or coherent (laser). The aim of this prospective study was to compare the performance of two monitoring systems, the Invos 3100 (Somanetics Corporation, USA) [5], which uses a diffuse light source and the more recently introduced Critikon 2020 (Johnson & Johnson Medical, UK), which uses a coherent light source. The systems were compared in terms of their ability to provide a stable measure of rSO_2, the variability of that measure and the agreement between measures provided by the two monitors in resting volunteers.

Methods

Local Ethics Committee approval was obtained in advance. Eighteen healthy volunteers, eight female and 10 male, aged between 21 and 30 years (mean age 27 years)

were recruited into the study. All gave informed consent. Subjects were seated comfortably and were asked to keep movement to a minimum during the recording period.

The Somanetics disposable adhesive sensor and the Critikon reusable sensor with disposable adhesive fixation pad were applied to either side of the forehead in accordance with the manufacturers' instructions. The placement sites were free of hair and situated as far above the supraorbital ridge and away from the midline as possible to minimise signal interference from underlying air or venous sinuses. The skin was cleaned and degreased prior to sensor application. A headband supplied by Critikon secured both sensors.

The signals from the oximeters were allowed to stabilise before starting data collection. The protocol was divided into two stages. The sensors were applied and steady-state cerebral oxygen saturation was monitored for at least 30 min. At the end of this period subjects were asked to hyperventilate until they experienced a subjective sensation of light-headedness, at which point they returned to their usual breathing pattern. Note was taken of the time of onset and termination of hyperventilation. The sensors were then resited so that each was placed contralateral to its original position and the above procedure was repeated. Although the Somanetics sensor is not designed to be reused, its adhesive properties were adequate for this single change in position. Data from both oximeters were logged continuously to a personal computer interfaced to the monitors at intervals of 15 s.

Comparisons were made of the ability of the monitors to provide stable readings of rSO_2 and of their relative performance in male and female subjects. Mean rSO_2 values were calculated for each oximeter for the first 30 min of recording. These values were used in a method comparison analysis [6] to assess the agreement (a) between oximeters running simultaneously and (b) within oximeters sequentially left and right. The mean change in signal observed during hyperventilation was analysed using the same methods in order to compare size and direction of the change in rSO_2 reported. When the bias between paired oximeter readings was not constant over the range of values observed, a correlated regression model was fitted to the bias using the generalised estimating equation (GEE) approach [7]. All analysis was carried out using S-plus for Windows 3.2, and the GEE models fitted using add-on functions supplied by V. Carey and A. McDermott of Harvard Medical School.

Results

Ten of the 18 volunteers participating in the study were successfully monitored for the full hour (eight women, two men, mean age 23 years). In one subject both systems recorded adequately for the first 30 min but, on relocation of the sensors, the Critikon failed to continue monitoring acceptable values. In two cases the Invos performed well, while the Critikon reported a poor or absent signal; in another two, neither oximeter was able to provide reliable readings. Three subjects were abandoned on the basis of Critikon signal problems without assessing the reliability of the Invos measurement. Thus, failure to obtain a stable reading occurred in eight out of 18 attempts using the Critikon and in two out of 15 using the Invos. The latter was found to work in two instances where the former failed but there were no comparable cases where the Critikon monitored successfully while the Invos did not. In all cases where one or both oximeters were unable to record adequately, the subjects were male; the Critikon showed a significantly higher failure rate in the male participants (Table 1, Fisher's exact test, $p = 0.0011$).

Table 1 Ability of Critikon 2020 to successfully monitor cerebral oxygen saturation.

	Critikon successful in monitoring?		
Subjects	**Yes**	**No**	**Total**
Female	8	0	8
Male	2	8	10
Total	10	8	18

A total of 10.5 h of data were recorded simultaneously by both monitors from 11 subjects (Table 2). A plot of the difference between the oximeter readings against their average value (Fig. 1) shows a clear trend for the bias to change with the underlying value of rSO_2. For rSO_2 values in the lower range the Critikon tends to read 5–10% higher than the Invos, with the reverse being true at high saturations. To explore possible causes of this phenomenon a GEE regression model was fitted, which takes account of the fact that there were two correlated measurements from each subject. There was no significant effect upon the bias of either the order of measurement (first 30 min vs. second

Table 2 A comparison of baseline readings.

	Mean rSO_2* at steady state (%)	
	Critikon	**Invos**
Range	64.0–71.3	56.7–75.3
Mean	68.0	64.3
Standard deviation	2.1	6.1

* Regional cerebral oxygen saturation.

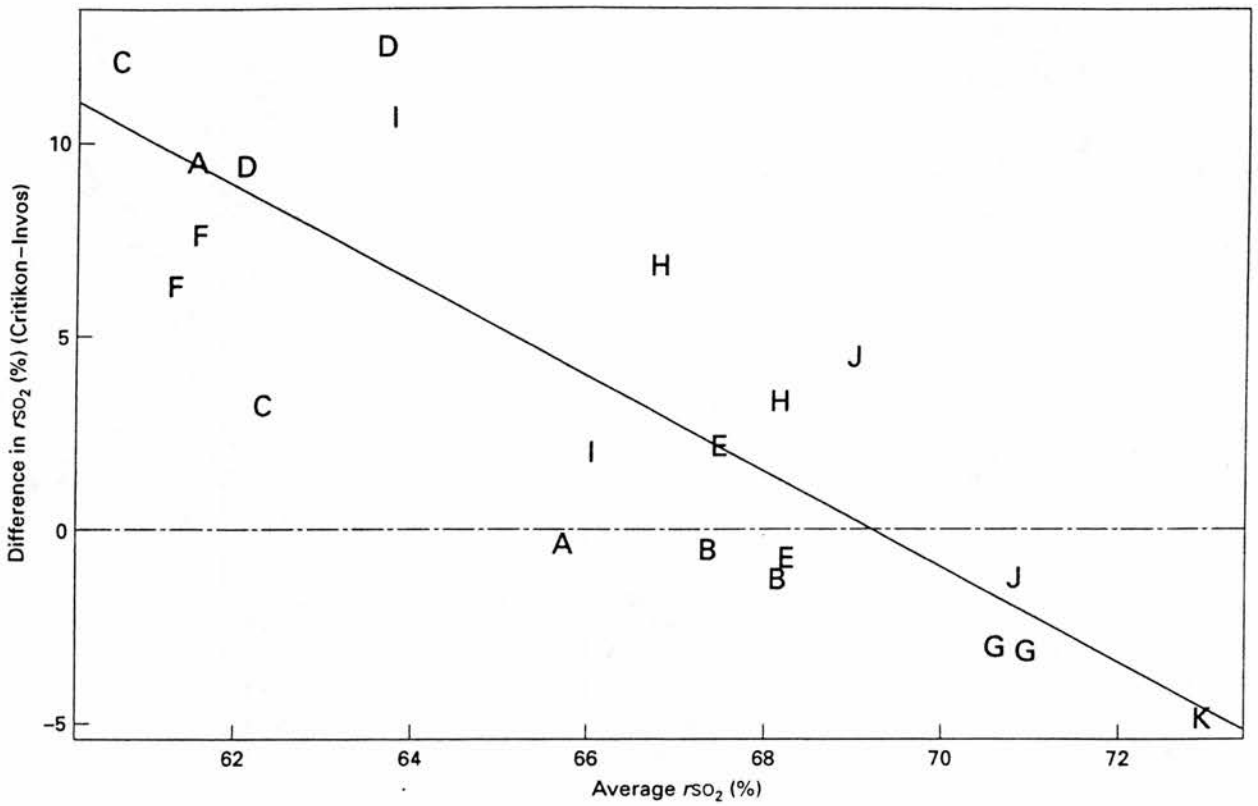

Figure 1 Differences between the regional cerebral oxygen saturation (rSO_2) shown by the Critikon and Invos monitors, plotted against their average, for each 30-min period. The letters A–K represent individual subjects.

30 min, $p = 0.657$) or the location of the oximeters (Critikon left vs. Critikon right, $p = 0.436$), but there was a highly significant relationship between the bias and the average rSO_2 (slope $= -1.22$, $p < 0.0001$). The fitted regression line is plotted on Fig. 1 and shows that, from the point of agreement at an rSO_2 of approximately 69%, for each percentage point decrease in rSO_2, the difference between the monitors would be predicted to increase by 1.22%. The estimated correlation between differences observed within the same subject was 0.133.

For the within-monitor comparisons, there was no apparent relationship between the side-to-side differences and the average value. The observed bias (right − left) for each monitor was not significantly different from zero (Critikon, mean $= -0.01$, $p = 0.985$; Invos, mean $= 2.08$, $p = 0.113$). The Invos, however, did show significantly more variability (F-test for homogeneity, $p = 0.0124$) and this is reflected in the limits of agreement, which are −3.3% to 3.3% for the Critikon and −6.1% to 10.2% for the Invos (Fig. 2).

For the hyperventilation phase of the experiment observed changes in rSO_2 ranged from −4.0% to 1.2% for the Critikon and from −9% to 1% for the Invos. Once again the size of the disagreement between monitors was related to the size of the change; the larger the average decrease in rSO_2 the larger the difference between monitors. A GEE regression model estimates the point of agreement at approximately −1% and predicts that for every 1% drop in rSO_2 the difference between the monitors (Critikon − Invos) will increase by 1.5% ($p < 0.0001$).

Discussion

Much controversy surrounds the validity of rSO_2 measurements obtained by reflectance cerebral near-infrared spectroscopy [8–10]. The original technique requires transillumination of the neonatal skull, where determination of the optical pathlength (the distance travelled by the photons from emitter to detector) is easily measured. This is not the case in adults, where the use of reflectance spectroscopy means that optical pathlength can only be estimated using complex modelling techniques (e.g. the Monte Carlo method [11]). The use of a coherent light source, with greater penetrance and less scatter, has theoretical advantages when using these models. In this manner the Critikon 2020, using laser optodes, should be superior to the Invos 3100, which uses a diffuse light

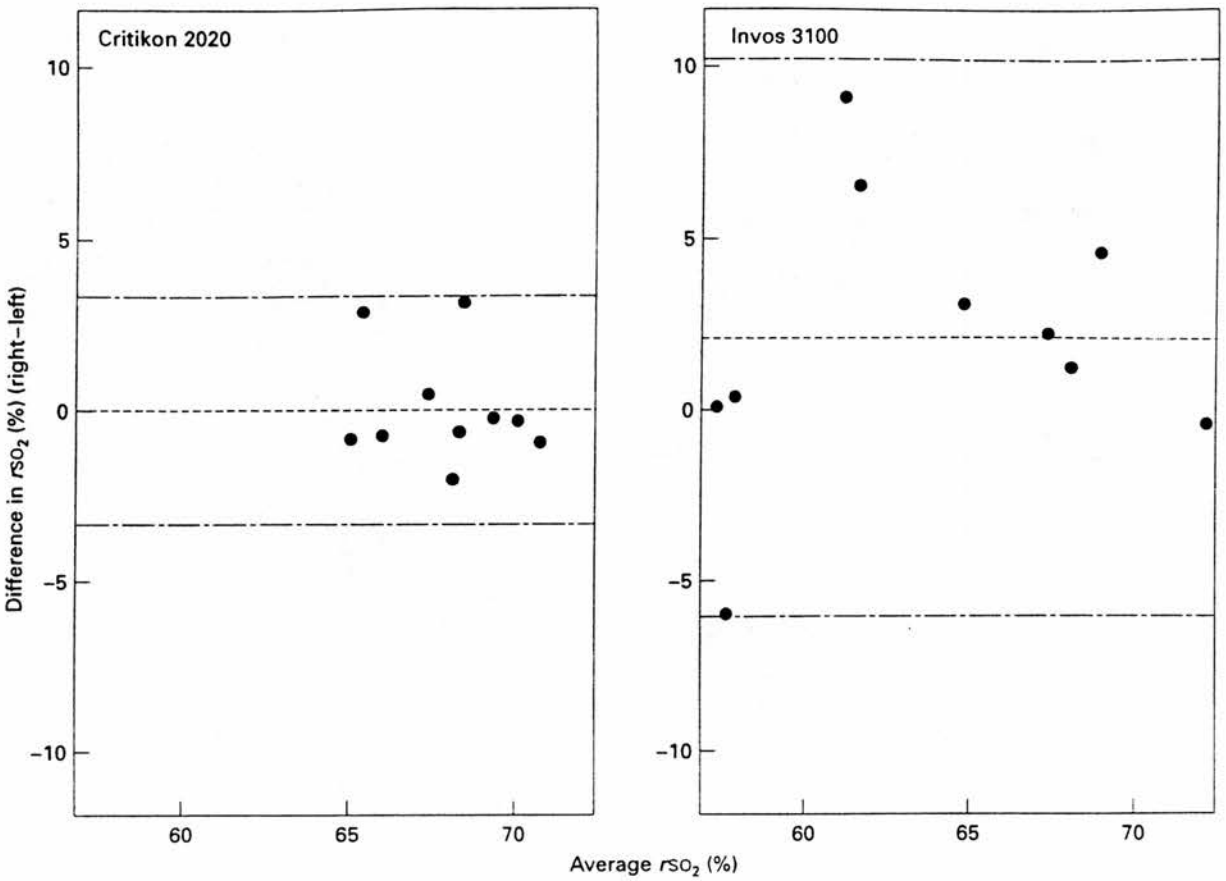

Figure 2 Differences between the average regional cerebral oxygen saturation (rSO_2) shown by each monitor for each 30-min monitoring period, plotted against their average for the total 60 min (– – – bias; – · – · limits of agreement).

source. A further and important difference between the two monitors is the choice of algorithm for quantification of rSO_2. The Critikon uses simultaneous solution of matrix equations of all chromophore absorption, whereas the Invos relies upon a questionable assumption of the compartmentalisation of the cerebral vascular bed.

Our original intention was to conduct this study on ventilated patients in the Intensive Care Unit and was motivated by several factors. Firstly, this technology has been developed for clinical settings such as intensive care where cerebral oxygenation is likely to become compromised and should therefore be assessed within that environment. Secondly, working with this population would have allowed a much longer total monitoring time per subject and minimised the effect of movement artefacts, shown to influence NIRS signals [12]. Finally, we intended to manipulate arterial oxygen saturation and partial pressure of carbon dioxide and correlate these changes with any alteration in rSO_2 reported by each of the oximeters. The insurmountable signal problems experienced with the Critikon instrument forced a radical revision of the original protocol. It failed to register an adequate signal from any of the four ITU patients to whom it was applied whilst simultaneous monitoring by the Invos instrument appeared to be consistent and stable. The decision to use healthy subjects was based on the finding that acceptable Critikon readings were only obtainable from this group.

Our results in volunteers demonstrate a higher failure rate with the Critikon than with the Invos. Although the Invos performed poorly in two cases, the Critikon also failed to record. It can be concluded that the Invos system is the more likely of the two to provide a recording that is continuous and stable. The incessant and frustrating 'poor signal' alarm that we experienced with the Critikon is totally unacceptable in a clinical environment.

We observed a strong positive association between failure of the Critikon monitor and male sex. Anatomical features of the head, for example skull thickness and mass of overlying muscle, are known to vary considerably between individuals and between different cranial regions [13, 14], which would support the hypothesis that the extent of infrared light scattering also shows inter- and intrasubject variability [15]. Adult women tend to have

smaller, lighter and thinner skulls than men [14]; the existence of special features, including the prominence of the supraorbital ridges and the contours of the forehead, which can be used to distinguish between male and female crania [16] is of particular relevance to the present findings. Further support for the observed sex difference is drawn from the suggestion that the amount of light collected by NIRS at a fixed space is greater for women than for men [15].

Assuming that a similar rSO_2 can be expected in equivalent areas of the two hemispheres in a healthy person, the side-to-side comparisons show that the Critikon monitor, despite the high failure rate, does appear to have significantly less inherent variability than the Invos. However, the dependence of the disagreement between the monitors upon the underlying value of rSO_2 suggests that either one or both of these machines is not giving an accurate value for regional cerebral oxygen saturation. A difference of up to 10% for values of rSO_2 in the range given by healthy volunteers does not bode well for clinical application of these monitors.

The values of rSO_2 from both monitors decreased during hyperventilation, as was expected, since systemic hypocapnia is known to result in cerebral vasoconstriction. Again the disagreement between the monitors varied with the average value, providing further evidence of the uncertainty of these values of rSO_2. However, without objective assessment of the change in arterial blood gas tensions it is difficult to analyse the significance of these changes. In the majority of cases the Invos showed the greater mean decrease in rSO_2, a finding that differs from a previous report in which it failed to respond to an episode of hypercapnia [9]. That study produced more detailed and direct measurements and it therefore seems plausible that the limitations of the present study design may be responsible for any discrepancies.

It is generally accepted that reflectance spectroscopy will become useful as a monitor of tissue oxygenation, and signal changes attributable to variations in cerebral oxygenation have indeed been demonstrated [12, 17, 18]. However, further development is required before this technique becomes commonly applied in the intensive care situation. The different algorithms used by the two systems assessed in the present study may explain some of the lack of agreement between them. We strongly suspect that deficiencies in sensor design may explain the unacceptably high failure rate in monitoring attributable to the Critikon instrument.

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